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Volume II

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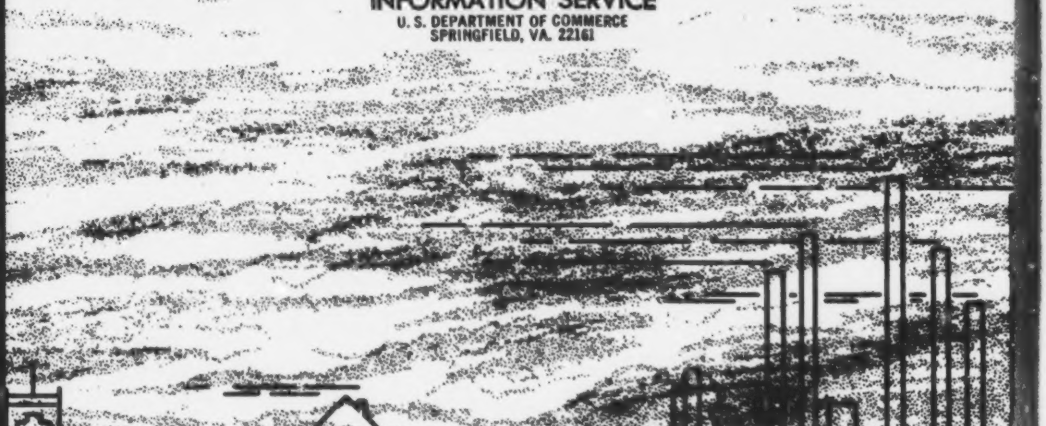

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16. ABSTRACT

This publication (in four volumes) contains the full proceedings of this international symposium. It is printed in the original language (Dutch, English, French, German, and Italian) of the individual reports or communications presented, with an English translation of the abstract of each report. The purpose of the meeting was to evaluate the methodologies and techniques available for establishing exposure-effects relationships for environmental pollutants with the goal of encouraging development of sound bases for the establishment of various approaches to protect the public health. These proceedings are made up of more than 250 scientific papers under the broad headings of: The scientific data base required to protect human health, experimental investigations, human effects studies, animal studies, metabolism interactions, indicators of exposure, population studies, toxicological studies, exposure monitoring, monitoring needs, experimental models, tissue measurements, and environmental measurements. Discussions following papers or groups of papers are also printed. An objective of the symposium was to encourage compatible methodology and data on scientific findings to help ensure that future data will be more directly applicable to the assessment of environmental health effects.

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RECENTI PROGRESSI NELLA VALUTAZIONE DEGLI EFFETTI DELL'INQUINAMENTO DELL' AMBIENTE SULLA SALUTE

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RELATION BETWEEN AIRCRAFT NOISE EXPOSURE AND HUMAN REACTIONS - A BIOLOGICAL MODEL

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ABSTRACT

The relation between aircraft noise exposure and the reactions in exposed populations is reviewed. Experimental data are presented which support the hypothesis that the peak of several noise levels is the determinant for the annoyance reaction in a community. The data also indicate the presence of a threshold for the perception of exposure frequency. The practical implications of the new principles are discussed and a model for human reactions to environmental noise is presented.

1. Introduction

Control of environmental noise constitutes an important part of the work required to make the modern world tolerable for man. **From a public health point of view a satisfactory control of environmental** noise sources requires a detailed information concerning dose-response relationships. In the control process the aim must be to use such relationships which are as acurate as possible and based upon the latest scientific knowledge. Only under such circumstances can adequate protection policies be formed and the technical investment always involved in environmental control be justified. This also implies that existing criteria and standards should be reevaluated in view of new progress in the field.

In the following the relation between aircraft noise exposure and the effect in the exposed populations is reviewed, in view of a new concept derived from experimental studies.

2. The noise exposure

A noise exposure includes several physical factors, such as the number of exposures, their duration, the frequency spectrum and the rise time of the energy front.

Several of these physical parameters are important for the development of an exposure reaction. Noise exposure indices have been developed, which contain different physical factors found to be relevant for the exposure effect. Indices are often constructed according to the acoustical principle of equal energy and contain expressions for the number of exposures as well as a mean of the noise level.

3. The exposure effect

The major effects of environmental noise are disturbance of ongoing activities, such as work or conversation, and interference with recreation or sleep (Alexandre [1]).

When the exposed individual experiences these effects they might be interpreted as an element of strain or stress which interferes with his well-being. The reaction will then be to express annoyance. Annoyance reactions are also influenced by extra-expositional factors, such as the socio-economical status, awareness of the exposure, habituation and psychological reactions on an individual basis. Due to the interference of these factors, the annoyance reaction in an individual is not well related to the level of exposure (Hazard [2]). The mean reaction in a group of individuals is better correlated to the exposure level as the influence of extra-expositional factors has been neutralized. From the public health point of view, the mean reactions must at present be used when annoyance reactions in a population are to be used as criteria of the exposure effect.

The presence of annoyance reactions can be assessed with the aid of social survey techniques, where the aim of the investigation is not revealed to the respondant. This masking effect is obtained by introducing questions on a variety of environmental annoyance sources in the questionnaire. Standard questionnaires have been proposed (OECD [3]).

For the description of the annoyance reaction the answers from the different questions on exposure effects have been combined into annoyance scores. The overall assessment of the annoyance by the individual can equally well be used to evaluate the mean reaction in a population group (Sõrensen et al [4]).

4. Annoyance due to aircraft noise

Many studies have been performed in different countries to evaluate the exposure effects of aircraft noise . In several of the studies an aircraft noise index has been developed using the annoyance data obtained in the particular investigation. An ideal dose-response relationship has been obtained by inserting weighting factors for the various physical components in the index.

Such an index must be considered as a hypothesis which has to be verified in renewed studies. When such studies have been performed, it has often been found necessary to adjust the original index by inserting other weighting factors than those originally developed (HMSO [5], Grandjean [6]). is in itself would cast doubt upon the validity of the procedure as such.

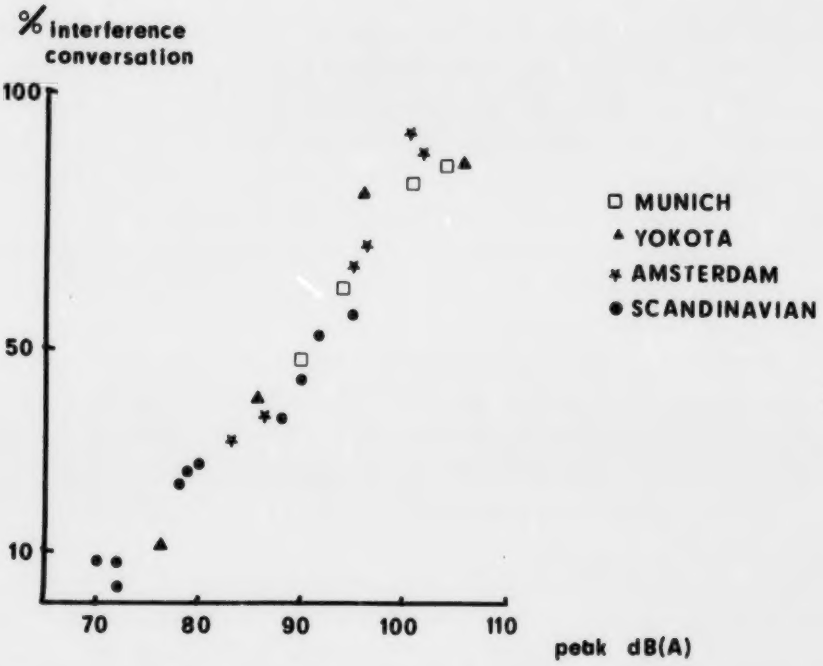

Figure I - Relation between conversation interference and aircraft noise exposure.

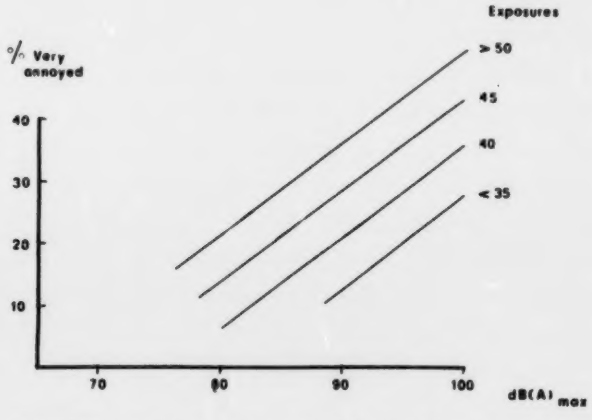

Figure 2 - Probable dose-response relationships for aircraft noise exposure and annoyance at different exposure frequencies.

A different approach concerning the expression of the noise exposure was taken in a Scandinavian investigation concerning aircraft noise annoyance performed 1969-1972 (Rylander et al [7]). In this investigation the number of aircraft movements and the noise level in dB(A) were kept separate and studied as independent variables. Variations were obtained by choosing investigation areas at different distances from the airport and by studying different airports with variations in the traffic.

The results from this investigation demonstrated that the number of exposures calculated as those equal to or above 70 dB(A) classified the areas in different exposure categories. Within each exposure category the extent of annoyance was closely related to the dB(A) level from the noisiest aircraft overflying the areas at least 3 times/ 24 hours. At about 50 aircraft exposures / 24 hours, a further frequency increase did not augment the extent of annoyance at equal dB(A) levels.

As the new principles imply important consequences from a public health point of view, a reanalysis program was undertaken with the goal to evaluate data from investigations performed earlier, using the principles developed in the Scandinavian study.

In the reanalysis program each area investigated was defined according to the total number of exposures (take-offs and landings) / 24 hours with a noise level equal to or exceeding 70 dB(A). The noise level was defined as the dB(A) level from the noisiest aircraft regularly using the airfield.

To allow for a comparison between the various studies the exposure reaction was expressed as interference with conversation, which is closely related to the general annoyance (Sörensen et al [4]). A detailed report of the findings will be published (Rylander et al [8]).

A summary of the results is illustrated in figure I for areas exposed to 50-174 overflights / 24 hours.

It is seen in the figure that a close dose-response relationship is present.

Data on areas exposed to less than 50 overflights / 24 hours are at present less complete, but a proposed dose-response relationship for such areas has been illustrated in figure 2 .

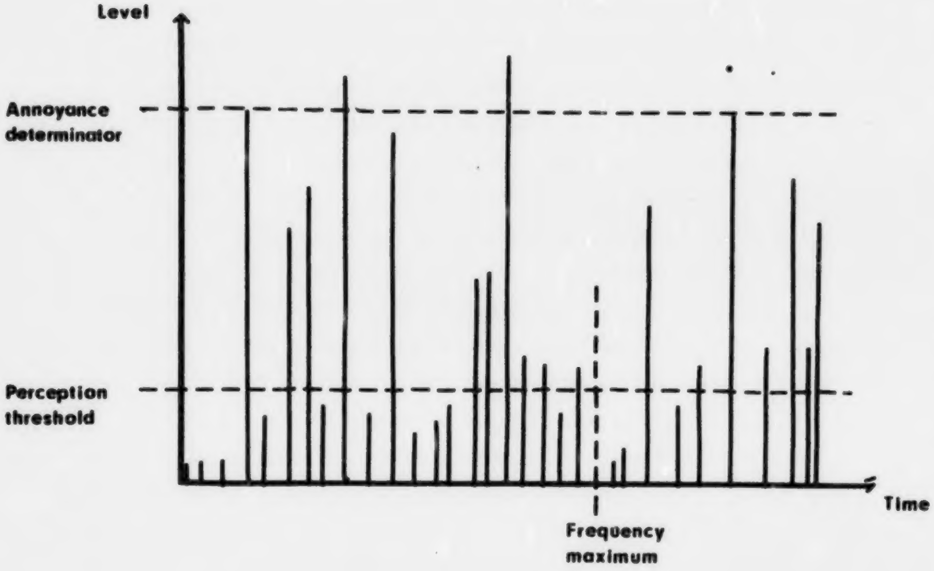

Figure 3
Proposed model for human response to environmental noise

5. Practical implications

When a standard concerning the accepted extent of annoyance in the community has been set, the critical noise contour around an airport is drawn using information concerning the dose-response relationship between the noise exposure and annoyance. This applies both to equal energy indices and the new principles. According to the latter, however, it is the dB(A) contour from the noisiest aircraft that determines the critical noise contour, irrespectively of the noise from less noisy aircraft.

The new principles for the relation between noise exposure and the development of effects in the exposed community makes it possible to construct a theoretical model for human noise exposure effects. This model consists of 3 determinants which are illustrated in figure 3:

(a) a perception threshold (for aircraft noise exposure levels equal to or more than 70 dB(A)

(b) an exposure frequency maximum (for aircraft noise about 50 exposures/ 24 hours)

(c) an annoyance reaction threshold based on peak exposure levels

The perception threshold and the exposure frequency maximum is probably influenced by the type of noise as well as the conditions under which the exposure occurs. This model could be valid also for traffic noise, noises in buildings and others. Investigations are in progress for the further exploration of these problems.

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DISCUSSION

SUTHERLAND (U.S.A.)

You spoke of 50 occurrences a day as a maximum frequency of disturbance. Could you please elaborate on this point?

RYLANDER (Switzerland)

For equal peak dB(A) values the annoyance will gradually increase as the number of exposures increases. At 50 exposures /24 hours a maximum is reached and a further increase in frequency will not influence the extent of annoyance. This conclusion is based upon data from 40 different countries with exposure frequencies ranging from 50 to about 400/24 hours.

TECHNIQUES FOR ASSESSMENT OF INDIVIDUAL SOUND EXPOSURE

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ABSTRACT

Objective and subjective methodologies were developed in a feasibility study to assess sound exposure of a person as he went about his usual daily activities. For the objective method, A weighted sound levels at the ear and two physiological parameters, heart rate and peripheral vasoconstriction at the ear lobe were monitored continuously on four subjects during their normal activity for one day. The instrumentation consisted of a small, portable package consisting of: (1) a subminiature electret microphone worn on the ear, (2) standard EKG chest leads for measuring heart rate, (3) a photoplethysmograph sensor mounted on the ear lobe for measuring peripheral vasoconstriction, and (4) signal-conditioning electronics connected to a small four-channel very low speed cassette tape recorder. Each of the four persons who participated in the experiment were instrumented early in the morning, wore the instrumentation for the entire day, and removed it at night. Each person also kept a log of location and activity during the day.

For the subjective part of the study, the components of human reaction to sound which have been studied extensively in the laboratory were evaluated in the real world by a questionnaire survey. This paper describes the application of one novel element of this questionnaire never before applied in this context. This consisted of a 24-hour diary using recall to probe for subjective impressions of dominant sounds for the previous 24 hours

of 100 subjects. Subjective ratings for a number of physical and psychological parameters of each of the hourly dominant sounds were also solicited. This provided a wealth of data on subjective response to sound on a time frame not generally available.

Results from this pilot test of these objective and subjective measurement techniques are presented to illustrate their interrelationship and usefulness for evaluation of overall sound exposure of individuals. Particular emphasis is placed on the ability of these techniques to properly assess the relative exposure and reaction of individuals to outdoor versus indoor or human sources of sound exposure. This study was sponsored jointly by the Department of Transportation and Environmental Protection Agency, USA.

1. INTRODUCTION

This paper presents some of the results of a pilot test program, funded jointly by the United States Department of Transportation and Environmental Protection Agency, of measurement techniques suitable for evaluation of the unwanted portion of the sound- or noise-exposure to which individuals are exposed as they go about their daily activity. The techniques evaluated were devised for potential application to a national survey of both objective and subjective measures of noise exposure and response of individuals.

2. OBJECTIVE MEASURES OF NOISE EXPOSURE AND RESPONSE

For the objective measures, four subjects were instrumented for one day each with a four-channel tape recorder to record continuously, (a) the envelope of A-weighted noise level exposure for each subject, (b) their heart rate by means of chest-mounted EKG electrodes, and (c) the relative blood flow rate by an ear lobe mounted plethysmograph sensor. The noise levels were measured with a miniature electret microphone located near the tragus cartilage of the ear and mounted on a light easily-fitted ear clip. Head diffraction was measured in a diffuse field and a maximum increase observed in diffuse field sound levels, before presence of the subject, 6 dB at a frequency of 3 kHz. The A-weighted noise envelope was split into a high level (70-110 dBA) and a low-level (35-70 dBA) channel to cover as wide a dynamic range as possible.

Subsequent tests indicated that the dynamic range should ideally have extended down to at least 25 dBA (to record quiet times at night) and up to about 120 dB (to record momentary high-level transients). For the heart rate signals, disposable EKG adhesive electrodes were attached to the chest wall. No discomfort was observed after at least 16 hours of continuous use. For the blood flow rate signals, a miniature gallium arsenide infrared light source was connected on one side of an ear lobe clip and opposite to an infrared phototransistor mounted on the other side of the ear lobe. Fluctuations in blood flow or changes in peripheral vasoconstriction were detected with this device. Use of an infrared signal minimized interference from normal light sources and variations in signal due to blood oxygenation changes which influence visible light transmission. All four channels of data were recorded on a miniature battery-driven cassette tape recorder operating at 2 mm/sec. Three dc-coupled channels with a 0 to 8 Hz bandwidth were used for the noise envelope and plethysmograph signals and an ac-coupled channel was employed with a 0.5 to 100 Hz bandwidth for the EKG signal. Subsequent signal analysis for this pilot test consisted essentially of playing back the tape onto a graphic recorder for visual and graphical analysis.

The graphic records of sound exposure at the ear of the four subjects exhibited similarities in some features and major differences in others. All four subjects exhibited a general pattern of high sound levels during daytime hours, strongly influenced by the rapidly fluctuating sound of one's own voice. During late evening hours, the exposure levels decreased as both outdoor and indoor activity decreased. Figure 1 illustrates the hourly variation in statistical levels for subject No. 3, a married 28 year old male, who was carrying out a variety of shopping trips and household chores on a Saturday. The detailed time histories of noise exposure for each of the subjects varied substantially as one might expect. Their individual life style or daily activity patterns result in a wide range in the type and severity of "encounters" with sound sources of all types, including, of course, many wanted or self-generated sounds. Figure 2 shows the approximate cumulative distribution in A-weighted sound levels over daytime hours for all four subjects.

The energy-average of the A-weighted sound level exposure over the daytime hours at the ear of the four subjects ranged from 69 to 78 dBA with an average of 74 dBA. As discussed in more detail in the full report from which this paper is taken (Sutherland, Braden and Colman [1]), the comparable energy-average daytime (0700-2200 hours) outdoor noise levels measured outside the subjects' residences ranged from about 55 to 66 dBA with an average of 62 dBA. The point is that, for the four subjects selected in this pilot study, the sound exposure at the ear was on the average and for the majority of the time, higher than outdoor noise levels at their residences. This difference is due to the subject's exposure to his own sounds, to non-residential sources, and to indoor residential sound sources.

The graphic records of variations in heart rate and relative blood flow rate demonstrated the feasibility of acquiring this type of data in conjunction with sound exposure records. However, no simple correlation was noted, nor was one expected, between sound exposure, heart rate and relative blood flow. More detailed, controlled, studies and analyses could be made, however, utilizing these techniques, to evaluate some of the non-auditory effects of noise exposure.

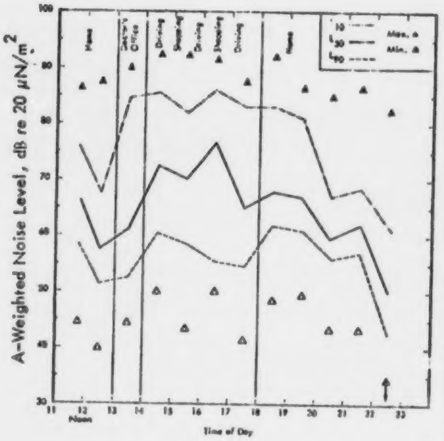

Figure 1. Hourly Variation in Statistical Measures of Sound Exposure at the Ear of Subject No. 3 on a Saturday

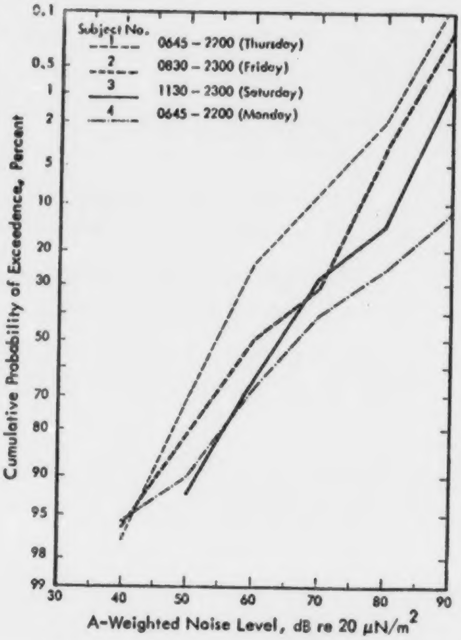

Figure 2. Comparison of Cumulative Sound Exposure Distributions Measured for Four Subjects During Daytime Period

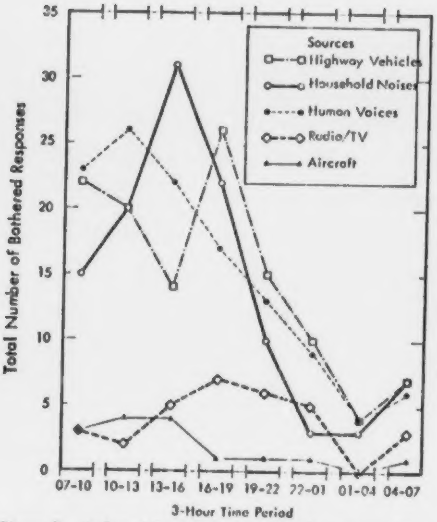

Figure 3. Bothered Responses Recalled Over Preceding 24 Hours, in 3-Hour Periods, for Five Major Sources of Sound

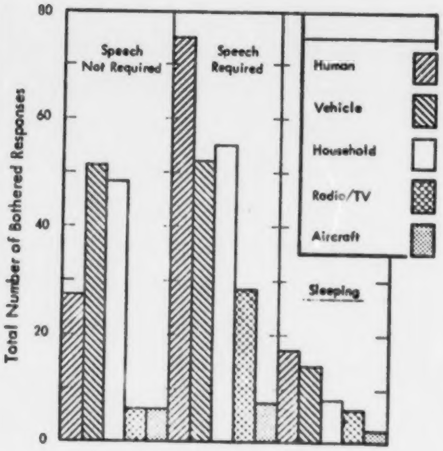

Figure 4. Bothered Responses Recalled Over 24 Hours Versus Three Activity Categories and Five Major Sources of Sound

3. SUBJECTIVE MEASURES OF NOISE EXPOSURE

The other element of the pilot program consisted of a field test of a detailed survey questionnaire designed to measure subjectively, among other things, the incidence and response to sound exposure events over the preceding 24-hour period – hour by hour. A recall technique was utilized in the questionnaire, administered to a total of 100 subjects selected at random (Kish [2]) from households located in three residential areas of Los Angeles. The patterns of recalled activity and location for the 100 subjects exhibited strong similarities to similar real time data recorded in diaries and reported in the recently published international study on the use of time (Szalai [3]). Figure 3 summarizes the total incidence of "bothered" responses in 3-hour periods to the dominant sounds recalled by the 100 subjects over the preceding 24 hours. The sources of these bothering sounds are grouped into five major categories. These type of data can provide a more accurate assessment of the relative significance of time of day on impact of outdoor noise sources. Note, however, that human voices and household noises, taken together, generated the majority of bothered responses for this pilot study. Figure 4 shows the same data (total bothered responses by all 100 subjects over the preceding 24 hours) broken down into three types of activity involved in which the "bothered" response occurred: (1) awake activity not requiring speech (i.e., solitary work or recreation), (2) awake activity requiring speech, and (3) sleeping. Note that "human" sound sources (i.e., voices, crying, etc.) were judged bothersome much more often during activity requiring speech than during activity not requiring speech. Surprisingly, however, no such difference was noted for "bothered" responses due to vehicle noises. This suggests that speech interference effects and "solitude interference" effects from outside noise sources may be comparable.

In summary, new techniques have been explored for obtaining objective and subjective measures of sound exposure of individuals. The results indicate they can be usefully applied to obtain more information about the influence of one's own activity or self-generated sound to properly evaluate the impact of one's encounter with external uncontrolled noises.

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DISCUSSION

BERLIN (Sweden)

What correlation do you think there is between physically measured noise levels and the effect on the central nervous system of the human being?

SUTHERLAND (U.S.A.)

Although our study did not attempt to answer this question, I believe the evidence from controlled laboratory studies of the effect of noise on the CNS is reasonably clear. Low level and complex effects are observed. However, it is very difficult to separate out any noise induced biological (or neurological) effects from those induced by other environmental factors.

DUPUIS (France)

What kind of work was the subject doing whose reactions are presented in Figure 3?

SUTHERLAND (U.S.A.)

The activities of the subject whose noise exposure is portrayed in Figure 3 consisted of a variety of outside activities including visits to a doctor's office, driving and shopping, followed, after 1800 hours, by activity in his home.

SLEEP DISRUPTION BY AUDITORY NOISE AND ITS EFFECT ON WAKING PERFORMANCE

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ABSTRACT

The research interests of our laboratory over the past four years have addressed 3 major questions. Firstly, is it possible to predict an individuals's response to auditory noise during sleep on the basis of how this individual responds to auditory stimuli during wakefulness? Secondly, is waking behavior affected when sleep is disturbed by auditory noise? and thirdly, if sleep disruption does influence waking behavior, what are the important parameters of this sleep disruption which produce the effects on waking behavior? Our experimental data indicate that the first two questions have relatively straightforward answers with the answer to the first question being no and the answer to the second question being yes. Specifically, the classic laws of psychophysics relating sound pressure level, auditory frequency, and psychological loudness during wakefulness are not applicable during sleep. Considering the carry-over effects of nocturnal auditory stimulation to waking performance, the results of our researches suggest that even minimal sleep disruption by auditory noise can be detrimental to certain waking behaviors. Turning to the parameters of sleep disruption which may produce these waking behavioral effects, our research indicates that morning performance deficits are more closely related to the total arousal which occurs during the night in response to the auditory noise and not necessarily the degree of arousal produced by the

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stimulus presentations per se. Considered together these data, along with other researches, tend to suggest that sleep is a qualitatively unique behavioral state necessary to an individual's well-being. And, should the normal functioning of this behavioral state be interfered with by auditory stimulation, even minimally interfered with, it is possible that optimal waking behavior may suffer. These results thus not only reflect certain fundamental aspects of sleep but also provide empirical support for the commonly held consensus that sleep disruption is one of the most serious problems of noise pollution.

Study supported by the National Aeronautics and Space Administration.

Introduction

The present report assumes the validity of the commonly held and empirically verifiable notion that sleep can be interfered with by auditory noise. From this starting point, we address three major issues. Firstly, is it possible to predict sleep disruption, i.e. arousal, from data obtained during the waking state? Secondly, are the disturbances produced by auditory noise during sleep restricted to the sleeping state or is there some carry-over degradation of waking behavior? And thirdly, if there is carry-over to the waking state, what parameters of nocturnal stimulation are most effective in producing this carry-over?

General Methodology

The general procedures which we employ [3,4,5] to investigate these issues involve human male volunteers between the ages of 18 and 35 who sleep for 3 or more nights within our laboratory bedroom setting. During each of these nights the individual's scalp-recorded electroencephalographic (EEG) activity is continuously monitored to provide both a measure of the subject's sleep as well as a dependent measure of the arousal produced by auditory stimulation. The auditory stimulation we generally employ consists of between 9 and 24 15-sec. stimulus presentations depending upon the requirements of the particular experiment. For obvious acoustical reasons, the auditory stimuli are produced by filtering white noise to exclude all frequencies save a 1/3 octave band centered on the frequency of interest.

The individual's response during sleep to these auditory stimuli is assessed in terms of a change in dominant frequency pattern of the subject's electroencephalographic activity. Virtually without exception, this change has been an increase of the frequency content of EEG pattern or what is traditionally termed cortical desychronization. This kind of response has been classically labeled as "arousal" by numerous researchers [1,6,9, 11] who have drawn the correlation between this sort of electrical activity and behavioral alerting. In all of the experiments which we will summarize, the assessment of the individual's EEG was performed by a digital computer system and, more recently, this has been upgraded to enable on-line, real-time analysis so that the stimulus presentations may be precisely related to the subject's ongoing pattern of sleep.

When demanded by the question under investigation, the subjects are required to perform a behavioral task before retiring at night and upon arising in the morning. The task is illustrated in Figure 1 and consists

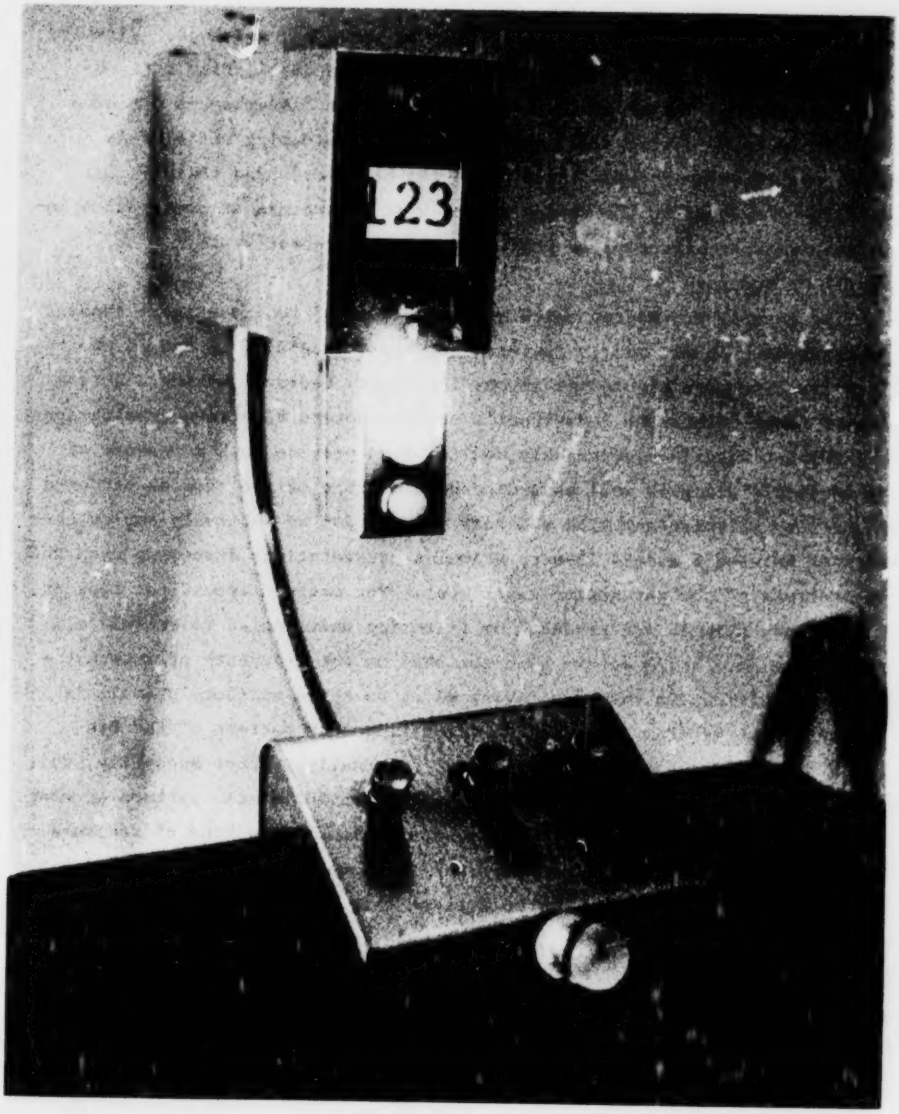

Figure 1. Photograph of short-term memory reaction-time task used to assess morning performance following sleep disruption.

of three individual stimulus lights and 3 response buttons. When a stimulus light is illuminated, the subject is required to press the appropriate response button and extinguish the light. The particular response button which extinguishes a particular stimulus light is determined by a 3-digit code presented in the window above the 3 stimulus lights. However, the code occurs with the first, and only the first, stimulus light for the series of stimulus light presentations that it is in effect -- i.e. between 3 and 10 stimulus light presentations. Thus, in order for the subject to respond most effectively, he must remember, on subsequent stimulus light presentations, which particular code is in effect. Each performance session is 10 min. in duration and the major dependent measure is response latency as measured from stimulus light onset to the subject's response to the correct button. We have also measured errors, percent errors, errors of commission, and percentage errors of commission but have not found these to be related to the occurrence of nocturnal sleep-disturbing auditory stimuli. Finally, it should be mentioned that in all of the experiments discussed below, we were interested in the effects of sleep disruption on performance and not acquisition. To realize this interest, each subject was required to come to the laboratory on each day of the week preceding his serving as a sleep subject to practice and become proficient at the operation of the behavioral task.

Prediction of Sleep Disruption

Using these procedures, we initially addressed the question of whether or not it would be possible to predict arousal during sleep on the basis of an individual's response to auditory stimulation during wakefulness. Briefly, the answer is no, at least, not during all types of sleep. Consider the waking psychophysical relationship between physical intensity, auditory frequency, and psychological loudness. Classically, the equal loudness contours presented by Pollack [8] and Robinson and Dadson [10] indicate that auditory sounds lower in frequency must be physically more intense than sounds higher in frequency if they are to be judged equally loud. Traditionally, the A-weighted dB scale has been used to approximate this psychological loudness function. To test whether or not the A-weighted dB scale would predict arousal during sleep, we presented sleeping subjects [4] with three types of auditory stimuli differing in frequency but equated for loudness by adjusting the intensity to 80 dB(A). On each night that

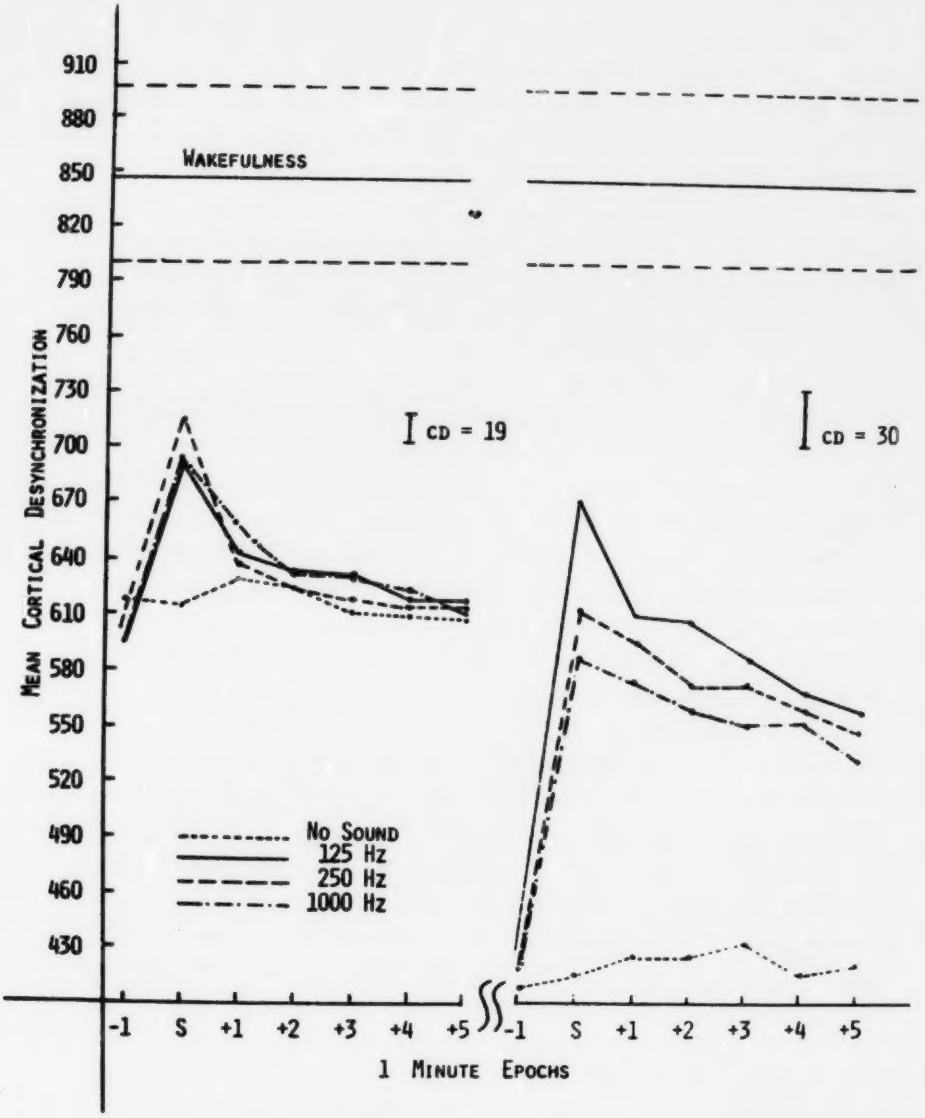

Figure 2. Mean error score (response latency in msec.) following sleep disturbed by broadband noise (JFO) and following undisturbed sleep (NJFO).

the subjects were presented auditory stimuli, only 1 frequency was used, i. e. either 125 Hz, 250 Hz, or 1k Hz. Moreover, there were 24 stimulus presentations so arranged that 12 of the presentations occurred when the subject's sleeping EEG exhibited predominantly fast-wave activity. The remaining 12 presentations occurred when the subject's EEG exhibited predominantly slow-wave activity. Sleep exhibiting these types of electrical activity have been traditionally, but inappropriately, labeled respectively light sleep and deep sleep. A fourth night that the subject slept in our laboratory was a no-stimulus control night. It should be pointed out and emphasized, that the sequential order of the 3 stimulus nights and the 1 control night was balanced acrossed the 8 individuals who served as the subjects for this experiment to control for "first-night" effects and order effects.

The results of these procedures are illustrated in Figure 2 which shows, on the left, the mean effect of the different auditory frequencies during sleep characterized by fast-wave EEG activity and, on the right, the mean effect of these same stimuli during sleep characterized by slow-wave EEG activity. As is apparent, and statistically verified by the critical difference computed from an analysis of variance, the three frequencies were equally effective during sleep characterized by fast-wave EEG activity. This would, of course, be predicted from the fact that the stimuli were equally loud relative to the A-weighted dB scale. However, from the family of curves presented on the right side of the figure, it is apparent that during sleep characterized by slow-wave EEG activity, the different auditory frequencies were not equally arousing. The data indicate rather that during this type of sleep, the subject is apparently more aroused by stimuli lower in frequency even though these stimuli are equally loud. Or put in another way, the subjects appeared more responsive to the sound pressure level of the different auditory frequencies during slow-wave sleep. The conclusion is that it would appear rather tenuous to attempt to predict the sleep-disturbing effects of auditory noise on the basis of waking data. It should be pointed out, in passing, that more recent research conducted in our laboratory [5] has exactly replicated these results using a more rigorous procedure where the subjects themselves equated the auditory stimuli for subjective loudness.

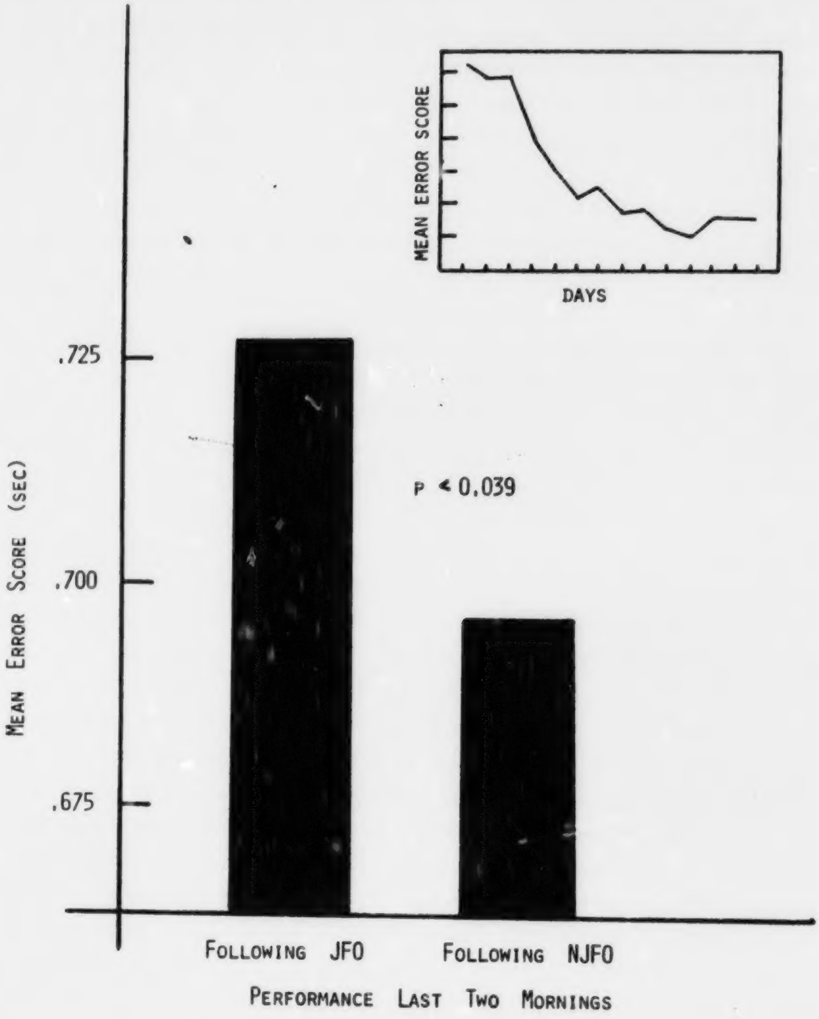

Figure 3. Arousal (mean cortical desynchronization) produced by three equally loud 80 dB(A) 1/3-octave bands of noise centered on the frequencies of 125 Hz, 250 Hz, and 1k Hz.

Carry-Over Effects

Turning to the question of whether these or similar sleep disturbances may carry over to influence waking performance, the answer apparently is yes. As an example, we may cite certain early research from our laboratory [3] involving 9 occurrences of a 15-sec. duration broad-band burst of auditory noise at a level of approximately 80 dB(A). These stimuli were evenly distributed over a night's sleep and the effects of their disruption of sleep assessed during morning performance of our reaction-time task. Figure 3 shows that there was a significant increase in mean response latency during the performance sessions following nights disturbed by auditory stimulation as compared to control nights when sleep was not disturbed. Since simple reaction-time tasks, as eloquently analyzed by Wilkinson [12], are usually insensitive to minimal sleep disturbances, our feeling at this time is that a critical parameter of our task is the requirement that the subject remember which code is in effect in order to maintain optimal performance. While this supposition is supported by similar other human [2] and animal [7] research, it is perhaps the intuitive appeal of the postulate which is most striking to us. That is, if sleep is to have any function, one function which would seem of importance is restoration. Since numerous data indicate that higher cognitive processes are quite susceptible to fatigue, then sleep may be the time when these processes are returned to optimal efficiency and any interference with sleep might then be reflected in the degradation of these higher cognitive and/or memory processes.

Sleep Parameters Affecting Waking Performance

Whether or not sleep disruption affects waking performance through higher cognitive processes or some other mechanism, the fact still remains that minimal sleep disruption can be detected as a decrement in certain waking performances. The final issue then which we have been concerned with is an attempt to provide some preliminary specification of the parameters of the nocturnal stimulation which may produce these carry-over effects to waking performance. As an initial, and more elementary query, we have questioned whether a large number of stimuli may be less debilitating on waking performance than a fewer number of stimuli. This might be speculated because the arousal produced by the individual stimulus occurrences, when a large number are presented, should be less. In other words, will habituation during sleep attenuate the disrupting effects of

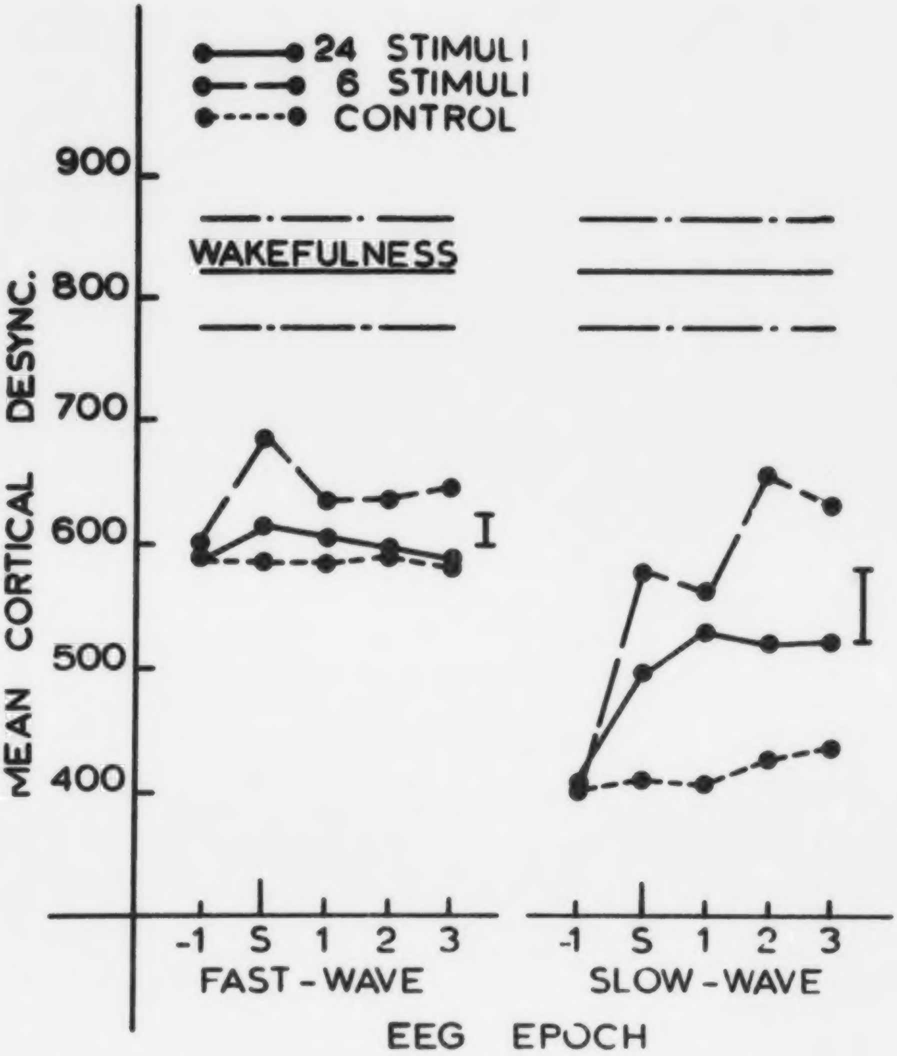

Figure 4. Effects of presenting no stimuli, 6 stimuli or 24 stimuli over a night's sleep in terms of mean arousal (cortical desychronization) produced by the stimulus presentations.

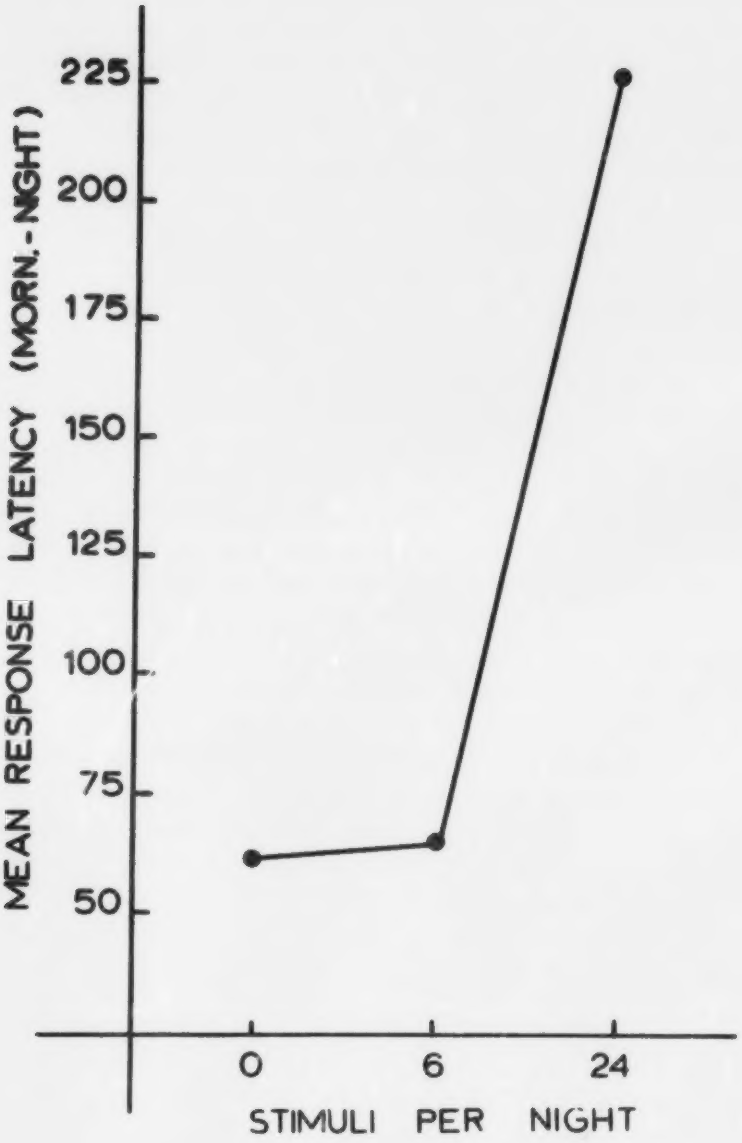

Figure 5. Morning performance (response latency) following undisturbed sleep, sleep disturbed by 6 stimulus occurrences, and sleep disturbed by 24 stimulus occurrences.

nocturnally presented noise? To address this question, we presented 6 subjects, on different nights, either no stimuli, 6 occurrences, or 24 occurrences of a 125 Hz burst of noise. Each stimulus presentation was 15-sec. in duration and had an intensity of 80 dB(A). The stimulus presentations were correlated with the subject's sleep pattern so that half of the stimuli occurred when the subject's EEG indicated fast-wave activity and half when the subject's EEG indicated slow-wave activity. The order of occurrence of the single no-stimulus control night, the 6-stimulus experimental night, and the 24-stimulus experimental night was, of course, balanced acrossed the 6 subjects. The arousal effects of the auditory stimuli *per se* are presented in Figure 4 and indicate the occurrence of a significant amount of habituation. That is, the mean arousal during both fast-wave sleep and slow-wave sleep was significantly greater when only 6 stimuli occurred during the night as compared to when 24 stimuli occurred during the night. However, as illustrated in Figure 5, even though the arousal associated with the individual stimulus presentations was significantly greater when 6 stimuli occurred, morning performance was significantly impaired only following the nights when 24 stimuli occurred. Thus, even though the arousal associated with the individual stimulus presentations was significantly less on the nights when 24 stimuli occurred, there was nonetheless a greater decrement (increase in response latency) during morning performance sessions following these nights. The conclusion is, of course, that one must not consider only the arousal produced by the occurrence of an auditory noise but one must also be cognizant of the number of stimuli which occur during the night. In fact, it may be that sleep disruption, as a determinant of waking performance, might more importantly involve the extent of the disruption in terms of distribution over a night's sleep than the degree of arousal in terms of whether or not the individual is awakened.

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DISCUSSION

Von DEPKA (Federal Republic of Germany)

Have your studies on sleep disruption of test persons revealed any information on the influence of noises in the same frequency spectrum, and if so, up to which noise level dB(A) are the influences relevant?

LEVERE (U.S.A.)

We have not, as yet, manipulated SPL or dB(A) level while holding frequency constant. Our major interest has been an attempt to establish certain psychophysical relationships during sleep which involve different frequencies when dB(A) is held constant. Our question has been whether the sleeping individual responds to auditory noise in a manner qualitatively similar to the individual who is awake - and apparently he does not. The questions you ask, while of great interest and importance, are more of a quantitive nature and at present outside our empirical framework.

IMPACT OF ENVIRONMENTAL NOISE ON SLEEP ELECTROPHYSIOLOGY AS MEASURED IN THE HOME

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ABSTRACT

In considering the effects of environmental noise on sleep, there are a number of limitations to laboratory studies. For example, it is not probable that the complexity of the naturally occuring environment can be accurately mimicked in the laboratory. In addition the generalizability of results from laboratory studies to the home environment is open to question; since, habituation to long-term noise exposure, such as several years duration, is not usually present in laboratory studies. Whereas, chronic high-level noise exposure is now the rule, rather than the exception, in urban life. In order to accurately record these chronic environmental effects, we developed a portable, economical, highly reliable system for recording electroencephalographic (EEG) electrooculographic (EOG) and acoustic signals. These signals are stored on tape and written out at the experimenter's convenience, or directly telemetered to the laboratory.

In considering the effects of environmental noise on sleep, there are a number of limitations to laboratory studies. For example, it is not probable that the complexity of the naturally occuring environment can be accurately mimicked in the laboratory. In addition the generalizability of results from laboratory studies to the home environment is open to question; since, habituation to long-term noise exposure, such as several years duration, is not usually present in laboratory studies. Whereas, chronic high-level noise exposure is now the rule, rather than the exception, in urban life. In order to accurately record these chronic environmental effects, we developed a portable, economical, highly reliable system for recording electroencephalographic (EEG) electrooculographic (EOG) and acoustic signals. These signals are stored on tape and written out at the experimenter's convenience, or directly telemetered to the laboratory.

Recording Procedure:

Subjects are recorded in pairs in order to minimize the number of tape recorders necessary. Each recording night, one hour before bedtime, a technician arrives at the subject's home, applies the electrodes, calibrates and turns on the equipment. Upon awakening, the subjects remove the electrodes and turn off the equipment. This system is easily tolerated by the subjects, and in fact, for long term studies, subjects can be taught to apply their own electrodes and operate the equipment without the constant attendance of a technician.

Equipment:

A modified four track analogue tape recorder acquires both the physiological and acoustic data. The physiological data consists of multi-plexed FM recordings of EEG and EOG, recorded on separate channels for the two subjects. The acoustic data consists of a continuous direct recording of noise exposure in the sleeping quarters. Time data is recorded on the remaining track.

We have applied this system to the study of aircraft flyover noise on human sleep directly beneath the flight pattern at Los Angeles International Airport. We have studied six couples for five consecutive nights in this naturally occurring environment, and five couples in a quieter area of Los Angeles. We have further studied, for fifteen additional nights, four of these couples, at a time in which the noise level in their homes was decreased during their sleeping hours due to a change in flight pattern.

Results:

Utilizing our technology, we have found, for example, that there is more "light sleep" in the noisy area as compared to the control area, as well as, more awakenings. "Deep sleep" was decreased in the noise condition and increased following the transition to a quieter environment. The subjects reported increased fatigue and tension and a lack of vigor in the noisy environment.

When an aircraft flyover occurred during light sleep - the normal transition to deep sleep was inhibited. When a flyover occurred during deep sleep, a transition to light sleep followed.

When the flight pattern changed and noise decreased at night, the previously disrupted internal cycling of sleep, returned to a more normal - smoother progression - of stages.

It is apparent then that intense noise exposure degrades the quality of sleep. In another area of research, we have accomplished long term studiesof sleep, where we recorded three nignts per week on 8 people for more than six months, indicating that chronic (in situ) monitoring of human sleep is feasible. Pressing environmental questions as to the public health hazards posed by chronic noise exposure,in naturally occurring environments, can now be assessed in a rigorous and objective manner, utilizing a reliable home recording system.

DISCUSSION

BOURDEAU (C.E.C.)

Parallel to your electrophysiological measurements, did you assess the subjective reaction of the persons under investigation?

FRIEDMANN (U.S.A.)

We gave our subjects the Profile of Mood Scale, the Stranfort Fatigue Scale and the San Diego Log to fill out. The subjects indicated they were more fatigued, more tense and had less vigor when subjected to noise.

BONNEFOUS (France)

Does noise, causing the disturbances which you spoke of, prevents sleep altogether or is it possible for light sleep without real rest to continue?

FRIEDMANN (U.S.A.)

The noise does not prevent sleep, as indicated by the EEG. However, that sleep is quite disturbed with many awakenings and is characterized by much more light sleep than is normal for 50-year old subjects.

CHAMBERS (Ireland)

Did you see much evidence of K-complexes in the EEG during the period of light sleep and during 'fly-overs'

FRIEDMANN (U.S.A.)

There was not an abnormal amount of K-complexes, nor did those which occurred appear to be in response to individual fly-overs.

NOISE INDUCED VEGETATIVE REACTIONS DEPENDING FROM AGE, SEX, AMBIENT NOISE, AND FROM A VASOACTIVE DRUG

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ABSTRACT

24 test subjects of different ages (12 males and 12 females) were exposed to noise of 105 dB(A) five times for 5 minutes each and intervals of 5 minutes between two noise periods. In the intervals there were ambient noise levels of 40 dB(A) and in another test series 70 dB(A). In addition we used in one half of the tests vasoactive drug (xantinol-nicotinacid = x.-n.). Peripheral blood circulation, pulse rate, breathing rate and temporary threshold shift were measured.

Our experiments resulted in different effects of noise in peripheral blood circulation and TTS, whereas no influence was observed concerning pulse rate and breathing rate. The results are discussed with respect to criteria and limits. It is recommended to look at the "whole reaction" of noise which may preferably occur in one of the following fields: otological, physiological and/or psychological reactions.

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1. Age and Moderator-Variables in Noise Tests

In recent research programs we tried to find out the importance of age concerning the effects of noise on human beings. Most of former tests have dealt with the age influence as a single parameter in noise experiments so that great standard deviations could be seen (OPPLIGER and GRANDJEAN [1], MATTHIAS and JANSEN [2] , LEHMANN and MEYER-DELIUS [3]).

There are occuring not only great variations within certain groups of age, but also great intraindividual variations. These intraindividual variations are due to the degree of initial value (WILDER [4]) and to other moderator variables. This means that in all research programs which deal with the influence of age it is necessary to give attention to intervening factors as much as possible.

2. Experimental Design of our Tests

24 healthy and good hearing test subjects were exposed to noise of 105 dB(A) in 96 tests. The age was between 19 and 58 years; the sex distribution was 12 : 12; in one half of the tests we used an ambiant noise level of 40 dB(A) and in the other half of 70 dB(A). In one half of the tests we applied a vasoactive drug: xantinol-nicotinacid (x.-n.) so that we resulted in "grouped test series".

We recorded aural and extraaural (vegetative) noise reactions, e.g. pulsamplitudes at the fingertips, pulse rate and breathing rate. We applied as a test noise white noise of 105 dB(A) which had a duration of 5 minutes and was applied 5 times; the intervals between 2 noise periods were 5 minutes; during this 5 minutes intervals we had in one half of the tests the ambiant noise of 40 dB(A) and in the other half of 70 dB(A). The vasoactive drug was applicated 125 minutes before the beginning of the noise periods. The dose was 600 mg x.-n. which was found out to be very moderate concerning the subjective response but was high enough to give a compensation of the noise effects as it was formerly demonstrated in several test series, (JANSEN, KLOSTERKÖTTER and REINEKE [5] , BERGMANN [6] , FEILER [7]).

3. Results

3.1 Peripheral Blood Circulation

In the different age groups we saw different reactions of peripheral blood circulation. As it was expected the sex groups showed different peripheral effects of noise as well as the different ambiant noise levels. In the same sense we recorded an increase in peripheral blood volume in both test series when the vasoactive drug was applied.

If all these influences are arranged and analysed in such an order as it is demonstrated in Fig. 1 different degrees of vegetative reactions can be observed. These reactions are very strong concerning the averaged reactions and the initial reactions in young male test subjects without drugs and in low ambiant noise levels.

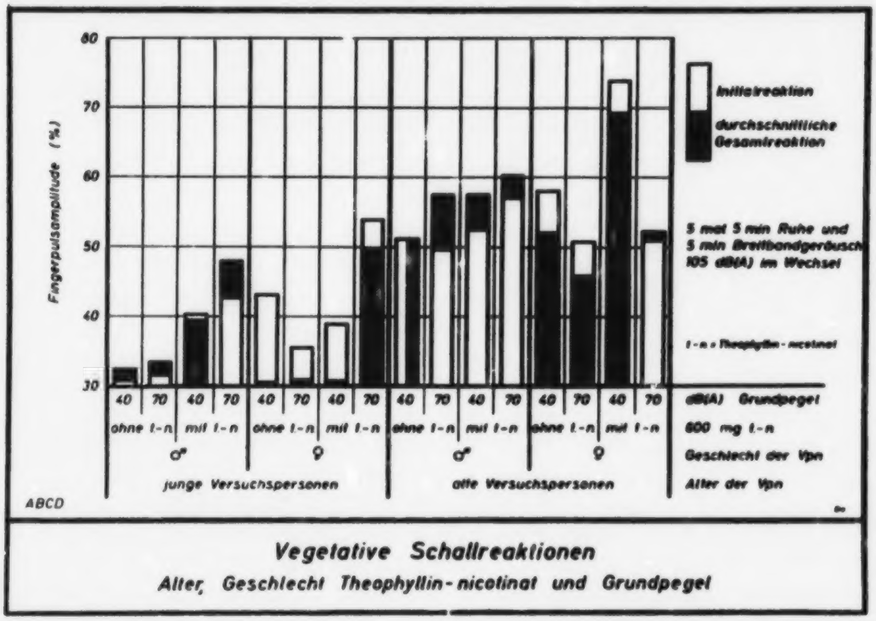

Figure 1

The least reactions are to be seen with old female test subjects after application of the vasoactive drug and in very low ambiant noise levels. One can conclude that young persons in noise situations react (averaged) in a higher degree of vasoconstriction than old persons.Young female persons show stronger vegetative reactions than young male ones whereas reactions in old male and female persons are almost the same.

Concerning the ambiant noise levels one can conclude from our experiments that the degree of "modulation" is decisive for the increase of vegetative reactions. However, old female persons show greater reactions in higher ambiant noise levels; it is supposed that female persons tend to show more "neurovegetative feelings" which may be responsible for those reactions seen here.

3.2 Other Vegetative Functions

Besides the factor peripheral blood circulation we recorded and arranged the results of the noise tests on pulse rates of our test subjects and of the breathing rates in the same manner as we did it in Fig. 1. We compared the pulse rate and the breathing rate before, during and after the noise tests in the different situations; moreover, we calculated for all situations the so-called "pulse-breathing-quotient" but we failed in finding any statistical significant difference between the situation without noise and with noise.

3.3 Hearing Measurements

We compared the hearing thresholds before and after noise exposures and resulted in different hearing thresholds similar to the results of influences on peripheral blood volume.

Fig. 2 is representing the slope of TTS2 in the different situations. One can see that young male test subjects without drugs and in low level noise situations show very high TTS by noise of 105 dB(A) whereas old female subjects with drugs and high ambiant noise levels show relative small TTS. The TTS of old aged is on the average 7.2 dB(A) smaller than that of the young ones. There is no difference between the

TTS after the drug application. The change of ambiant noise level is without any effect on TTS with young persons and old persons show only small differences between high and low ambiant noise level.

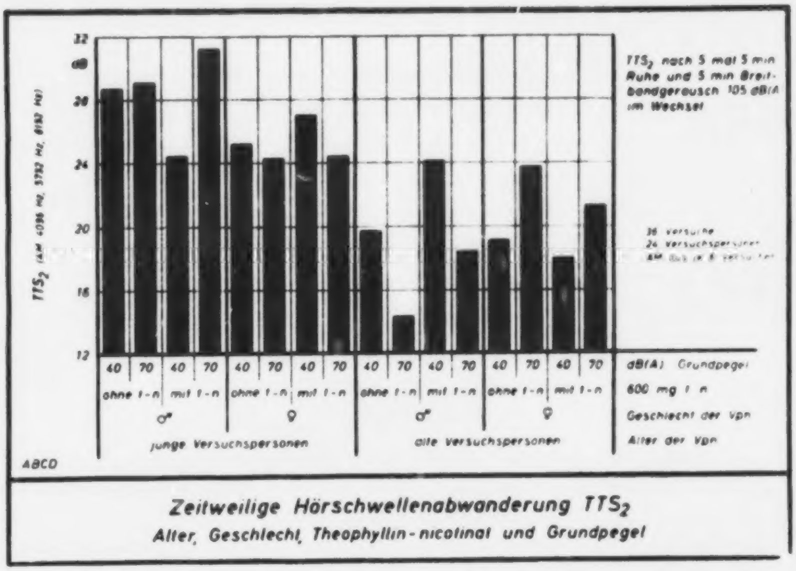

Figure 2

4. Discussion and Conclusion

Comparing the noise situations and the factors, influencing the degree of body reactions one can draw the conclusion that a distinct degree of reaction is related to certain groups of human being, so that "group-specific-answers" in typical noise situation are expected. From the standpoint of prophylactic medicine one must be aware of the fact that population groups may show increased effects on circulatory system and/or losses in hearing acuity. For the single person the absence of hearing effect in noisy situation does not mean that there is no other reaction; this reaction might be found in disturbances of vegetative functions.

The recommendation of criteria and especially for limits should therefore - according to our experiments - be given with respect to those who have the greatest bodily reactions and this group is in our experiments the group of younger people. As it was demonstrated older people are not so much disturbed by noise concerning the physiological reactions. But we know that the psychological disturbance by noise is much greater in older people than in younger people. This may be due to the decreasing ability of the older human organism to process and assimilate the noise stimuli as young people are able to do. The greater psychological disturbance might be assessed as a compensatory effect which must not be evaluated less than the physiological responses.

If this idea is significant the objective and measurable physiological noise effects in young people might be in the same way representative for old people who show no or smaller vegetative reactions.

We always have to consider the "whole reaction" in a human being, which may preferably occur in one of the great fields: otological, physiological and/or psychological reactions.

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UNTERSUCHUNG DER WIRKUNGEN AUF DEN MENSCHEN
HUMAN EFFECTS STUDIES
ETUDES DES EFFETS SUR L'HOMME
STUDI DEGLI EFFETTI SULL'UOMO
ONDERZOEKINGEN NAAR EFFECTEN BIJ DE MENS

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(Bundesrepublik Deutschland)

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CONCENTRATION DU PLOMB DANS L'EAU POTABLE ET PLOMBEMIE D'UNE POPULATION ADULTE

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RESUME

L'agressivité de l'eau potable, délivrée dans une grande partie de l'agglomération verviétoise, est responsable d'une lente dissolution des canalisations en plomb qui équipent encore la moitié des habitations. L'enquête épidémiologique, menée sur un vaste échantillon (± 1200 personnes), permet de préciser l'importance de l'imprégnation saturnine de cette population exposée à de très petites quantités de plomb pendant des périodes prolongées. Les résultats sont significatifs: le groupe exposé au plomb présente une plombémie moyenne deux fois supérieure à celle des groupes témoins. On a étudié la répartition des plombémies en fonction de quelques variables comme l'âge, le sexe, et l'habitude de fumer. On donne également les premiers résultats bruts d'une enquête sur la mortalité au cours des dix dernières années dans la même région. Cette dernière paraît beaucoup plus influencée par le caractère de l'eau douce que par la présence de traces de plomb.

ABSTRACT

The corrosive properties of the drinking water supplied to a large part of the Verviers city area have given rise to the

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slow dissolution of the lead pipes still in use in half the dwellings. The epidemiological survey, carried out on a huge sample (± 1200 persons), enables us to determine the lead levels in this population group exposed to very small quantities of lead for long periods. The results are significant: the group exposed to lead shows an average blood lead level double that of the control groups. Blood lead levels were studied as a function of certain variables, such as age, sex and smoking habits. The first, uncorrected, results of a survey on mortality in the same region during the last ten years are also available. The mortality appears to be influenced far more by the nature of the soft water than by the presence of trace quantities of lead.

1. Introduction.

On admet aujourd'hui que l'eau alimentaire et les aliments surtout lorsqu'ils sont confectionnés avec des eaux plombifères, constituent les principaux agents de l'augmentation de la charge corporelle en plomb. Nous avions déjà montré qu'il existait en Belgique une région à population dense où la situation était à ce point de vue particulièrement défavorable. L'eau très peu minéralisée et acide de la rivière Gileppe est utilisée comme eau potable dans la ville de Verviers et dans certaines parties de l'agglomération verviétoise; elle provoque la mise en solution du plomb des canalisations. Les quantités de plomb libérées peuvent aller jusqu'à plusieurs mg/l suivant l'état des canalisations, leur âge et les modes d'utilisation de l'eau potable. Deux à trois mètres de tuyau suffisent à provoquer la mise en solution d'une quantité de métal supérieure aux normes de l'OMS alors que d'autres eaux de même origine mais traitées chimiquement ne dissolvent pratiquement pas de plomb.

Dans le but de dégager les conséquences biologiques de l'ingestion continue de plomb pendant plusieurs dizaines d'années, nous avons réalisé une enquête épidémiologique sur le taux de plomb sanguin des habitants de cette région. Nos résultats portent sur environ 1200 adultes des deux sexes. Ce nombre élevé permet d'étudier statistiquement les corrélations éventuelles existant entre le paramètre biologique et divers paramètres caractérisant les individus (âge, sexe, etc) et la qualité de l'eau potable consommée.

2. Choix du test biologique.

Le dosage du plomb sanguin constitue un indice significatif d'une absorption de plomb. Bien que simple, le dosage présente cependant des problèmes techniques qui influencent directement la reproductibilité des résultats. Il a été réalisé par absorption atomique classique après défécation du sang à l'acide trichloracétique, complexation du plomb dans le surnageant par du diéthyl-dithio-carbamate d'ammonium et de pyrrolidine et extraction du complexe à la méthyl-isobutyle cétone en milieu acide. La reproductibilité de la méthode est voisine de 10% pour des plombémies de 10 µg/100 ml et de 5% pour des plombémies de 20 µg/100 ml. Ce léger défaut de précision de la méthode est compensé par l'effectif de la population étudiée.

3. Choix d'un groupe de population.

L'échantillon que nous avons sélectionné devait répondre à différents critères : le nombre d'individus étudiés devait être élevé (supérieur à 1000), ces individus devaient être en bonne santé, répartis de façon homogène selon le sexe et l'âge, non exposés professionnellement au plomb et avoir vécu pendant les cinq dernières années dans un environnement identique. Les donneurs de sang de la région verviétoise répondaient de façon satisfaisante à la plupart de ces exigences. On a pu ainsi définir dans l'échantillon un groupe exposé au risque et des groupes témoins vivant dans un environnement identique, différenciés uniquement par la teneur en plomb de l'eau potable.

Notre premier travail avait montré que l'acidité de l'eau agissait directement sur la mise en solution de quantités plus ou moins importantes de plomb dans l'eau alimentaire à partir des canalisations.[1, 2] Le même schéma a été suivi pour l'étude de la plombémie. La population étudiée est répartie en quatre groupes :

a. Ceux dont l'eau potable est douce et dont les canalisations à l'intérieur des maisons comportent plus de 2 mètres de tuyau de plomb.

b. Ceux qui reçoivent de l'eau douce par des canalisations comportant moins de 2 mètres de tuyau de plomb ou en matériaux autres.

c. Ceux qui reçoivent de l'eau dure (ou durcie) par des canalisations en plomb.

d. Ceux qui reçoivent de l'eau dure par des canalisations en matériau autre.

4. Résultats.

Le tableau 1 donne les plombémies moyennes mesurées dans chaque groupe, leur écart-type et l'effectif des groupes. On peut conclure à la lumière de ces chiffres que si la plombémie moyenne de la population exposée au risque (groupe a) n'atteint pas des niveaux dangereux, elle n'en traduit pas moins une imprégnation significative de l'organisme par le toxique. Le grand nombre de déterminations effectuées permet d'analyser dans le détail quelques facteurs d'influence.

	Définition du groupe	Plombémie µg/100 ml	Ecart-type	Populations étudiées
Gileppe	Eau douce Tuyaux de plomb	26,9	15,8	294
	Eau douce Tuyaux autres	20,4	13,4	443
Eupen Bilstain Fierain Captages	Eau dure Tuyaux de plomb	14,9	11,0	120
	Eau dure Tuyaux autres	13,3	8,6	261

Tableau 1 : Plombémies moyennes de la population étudiée classée en quatre groupes.

4.1. La combinaison de la qualité de l'eau et de la nature des canalisations joue le rôle primordial. Dans les communes où l'origine de l'eau potable est multiple, donc sur des populations plus restreintes, on retrouve les mêmes écarts significatifs de façon presque constante même lorsqu'on ne tient pas compte de la nature des canalisations.

	Eau douce*		Eaux dures**	
	Nombre de Prélèvements	Plombémie moyenne	Nombre de Prélèvements	Plombémie moyenne
Origine Unique				
Verviers	354	24,7	-	-
Ballice	-	-	42	12,6
Origine multiple				
Andrimont	35	27,3	24	14,5
Dison	54	23,0	29	15,7
Dolhain-Limbourg	27	25,5	7	15,0
Ensival	66	23,7	26	15,0
Heusy	53	17,4	9	15,5
Petit Rechain	17	15,3	3	7,3
Stembert	34	15.1	10	15,9

* provenance : Barrage de la Gileppe

** provenance : Barrage de la Vesdre (Eupen) et captages divers.

Tableau 2 : Comparaison des plombémies, en µg/100 ml des habitants de quelques communes verviétoises ou parties de communes selon l'origine de l'eau potable.

Ainsi les habitants d'une même commune, vivant dans des conditions de vie et d'environnement identiques (nourriture, pollution atmosphérique, occupation journalière,...), présentent des plombémies moyennes qui varient suivant la provenance de l'eau. L'écart relatif observé varie d'un endroit à l'autre : il est lié à la nature et à l'âge des différentes parties de l'agglomération (les faubourgs les plus récemment urbanisés ne sont plus équipés de tuyaux en plomb).

A l'intérieur du groupe a exposé au toxique, nous enregistrons les résultats suivants d'après la nature des canalisations :

- A Verviers, les personnes habitant dans des immeubles équipés de canalisations en matériaux autres que le plomb présentent une plombémie moyenne pratiquement identique à celle des populations témoins desservies par des eaux

Tableau 3

- PLOMBEMIE MOYENNE* DES HABITANTS DES COMMUNES OU PARTIES DE COMMUNES UTILISANT EXCLUSIVEMENT DE L'EAU DOUCE, EN FONCTION DE LA NATURE DES CANALISATIONS

Matériau / Communes	Plomb		Acier galvanisé		Cuivre		PVC	
	n**	Plombémie	n**	Plombémie	n**	Plombémie	n**	Plombémie
Andrimont	19	33,7	-	-	11	23,1	5	12,4
Dison	27	29,4	3	15,6	19	17,4	5	14,2
Dolhain	17	32,6	-	-	6	22,3	4	16,5
Ensival	38	27,3	-	-	20	20,0	8	14,1
Heusy	21	21,5	-	-	22	16,3	10	11,2
Petit Rechain	7	22,3	2	13,0	5	8,8	3	11,7
Stembert	6	29,8	-	-	18	9,8	10	14,8
Verviers	131	31,3	5	12,8	73	18,2	20	15,2
Total	266	29,8	10	13,7	174	17,4	65	14,4

* en µg/100 ml
n** nombre d'échantillons de sang analysés

moins acides (environ 15 µg/100 ml) et à celle des populations étudiées dans la plupart des pays voisins. Elle correspond à l'exposition normale au plomb qui résulte, dans nos régions, des aliments ingérés et de l'air inhalé.

- Lorsque l'eau douce de la Gileppe est distribuée dans une canalisation contenant 2 mètres ou plus de tuyau de plomb, ce qui représente un peu plus de la moitié de l'échantillon, on enregistre une plombémie moyenne de 30 µg % soit le double des témoins. La différence est significative. Pour autant que la résorption et l'élimination du plomb soient comparables dans les groupes examinés (ce qui paraît vraisemblable a priori vu les caractéristiques des populations étudiées), on peut considérer que les personnes du groupe exposé vivent dans des conditions qui se traduisent biologiquement par un doublement de la plombémie et sans doute de la charge corporelle totale en plomb. On peut dès lors supposer que ce groupe de population subit depuis plusieurs dizaines d'années, au point de vue sanitaire, une situation comparable à celle qu'on rencontrerait dans nos villes suite à une augmentation de la concentration en plomb atmosphérique. On peut rappeler à ce titre qu'une plombémie de 30 µg % a été enregistrée en 1961 chez les employés d'un tunnel routier à Boston[3] qui voit défiler un peu plus de 35.000 véhicules par jour (l'air évacué du tunnel contenait jusqu'à 45 $\mu g/m^3$ de plomb).

La moyenne élevée de la plombémie de la population exposée ne traduit pas seulement des plombémies plus élevées mais également une fréquence plus grande des plombémies supérieures au taux d'imprégnation établi par Zielhuis à 40 µg % (5. fréquence est de 34,4% dans le groupe exposé et est pratiquement nulle dans les autres. On peut donc évaluer à 35% la probabilité pour les individus exposés au risque de ne pas réussir à excréter en continu les quantités de plomb ingérées et de voir ainsi leur plombémie augmenter lentement d'année en année. Cette probabilité augmente vraisemblablement en fonction de la longueur du métrage en plomb de la canalisation mais elle n'a pas été calculée.

	Groupe Gileppe (canalisations en Pb).		Groupe Témoin	
	Population	Plombémie	Population	Plombémie
Hommes	152	29,9	211	15,0
Femmes	77	24,6	114	11,1

Tableau 4 : Influence du sexe sur la plombémie (en µg/100 ml).

4.2. L'influence du sexe est statistiquement significative : les plombémies sont légèrement supérieures chez les hommes, témoins et exposés. Bien que la différence ne soit pas très marquée, on constate qu'elle va dans le même sens et avec une amplitude identique à celle rencontrée dans d'autres enquête menées à Milan[4] et aux Etats-Unis[3].

a) Dans le groupe exposé au toxique.

Age (ans) Nombre de cas	20	21-30	31-40	41-50	51-60	61-65
Hommes, 265 cas	20	22	26	24	28	31
Femmes, 148 cas	9	10	23	19	25	23

b) Dans les groupes témoins.

Age (ans) Nombre de cas	20	21-30	31-40	41-50	51-60	61-65
Hommes, 136 cas	11	13	14	15	15	16
Femmes, 81 cas	-	6	10	10	14	11

Tableau 5 : Variation de la plombémie moyenne (µg/100 ml) en fonction de l'âge.

4.3. L'influence de l'âge est détaillée dans le tableau 5. Tant pour les hommes que pour les femmes des groupes témoins, on n'enregistre pas d'accumulation importante du plomb sanguin au-dessus de trente ans. Il semble en équilibre stable. Dans le groupe exposé, on enregistre une absorption et une accumulation du plomb qui déplacent ainsi, de manière presque continue, l'état d'équilibre qui gère habituellement les entrées et les éliminations du toxique. Malgré la dispersion des points expérimentaux, on peut souligner une différence d'évolution entre les deux sexes. On constate qu'à 20 ans, les hommes présentent une plombémie plus élevée que les femmes mais la plombémie de celles-ci évolue plus vite que celle de leurs compagnons. Il serait intéressant de rechercher la raison de ce phénomène et de la relier à des facteurs intrinsèques ou extrinsèques définis. Une étude différentielle selon les sexes sur les mécanismes biochimiques d'accumulation, de transfert, de stockage et d'élimination du plomb pourrait apporter un début d'interprétation à ces résultats (influence du métabolisme du calcium par exemple).

5. Etude de la mortalité.

Les études de mortalité constituent l'outil le plus simple, même s'il n'est pas le plus adéquat, dans l'étude de nombreux problèmes de santé publique. Nous avons donc tenté de réunir quelques premiers chiffres à partir de la statistique nationale des causes de mortalité, telles qu'elles ont été classées sur base des certificats de décès recueillis de 1960 à 1971 dans la ville de Verviers (eau douce), dans la ville d'Eupen (eau de même origine, mais durcie) et pour l'ensemble de la Belgique (population de référence). On n'a pas repris dans le tableau les mortalités par affections transmissibles et par causes accidentelles. Bien que ces chiffres n'aient pas encore été soumis à un indispensable traitement statistique et qu'ils portent sur une population restreinte, on peut déjà voir que la mortalité par cardiopathies et maladies cardiovasculaires est fortement marquée par la composition de l'eau. Cette conclusion est déjà classique et ne sera pas développée ici. Par contre, même en considérant ces chiffres comme approximatifs, on ne discerne pas d'influence attribuable au plomb : la fréquence des mortalités par néphrite notamment ne semble pas affectée tandis que celle des cirrhoses pourrait l'être. Afin de pallier ce manque relatif d'information sur les conséquences lointaines de l'intoxication à très bas bruit, l'enquête se poursuit actuellement en déterminant, sur les mêmes individus, les paramètres biologiques habituellement associés à l'intoxication saturnine. Leur étude montrera comment ils varient dans une population exposée de façon homogène à un toxique à faible concentration.

Causes de Décès.	Catégorie	Verviers (34.000 hab.)	Eupen (15.000 hab.)	Etat Belge (10.000.000hab.)
Anémie	B23	61,7	60,0	23,7
Rhumatisme articulaire aigu	B25	2,9	0	3,6
Cardiopathies rhumatismales chroniques	B26	61,7	26,6	34,2
Maladies hypertensives	B27	326,0	286,0	264,0
Maladies ischémiques du coeur	B28	2767,6	820,0	1696,0
Autres cardiopathies	B29	2138,2	1433,3	1341,7
Maladies cérébro-vasculaires	B30	2098,1	1400,0	1320,7
Cirrhose du foie	B37	185,2	146,6	112,3
Néphrite et néphrose	B38	132,3	200,0	100,6
Complications de la grossesse et de l'accouchement	B41	23,5	26,6	4,3
Anomalies congénitales	B42	82,3	6,6	100,0
Lésions obstétricales, états anoxémiques ou hypoxémiques	B43	50,0	26,6	60,8
Autres causes de mortalité périnatale	B44	364,1	113,3	176,4
Etats morbides mal définis	B45	2205,8	1333,3	973,1
Autres maladies	B46	455,8	333,3	2228,5

Age moyen des décès compris entre 65 et 70 ans; sauf pour le groupe B42, B43, B44 (mortalité périnatale) et le groupe B45, B46 (sénilité).

Tableau 6 : Mortalité spécifique selon les certificats de décès dans deux villes de l'est de la Belgique et pour l'ensemble du pays, par 100.000 habitants et pour la période 1960 à 1970.

6. Conclusions.

L'imprégnation saturnine de la population verviétoise alimentée avec une eau potable douce et agressive distribuée à l'intérieur des maisons par des canalisations en plomb est double de celle de trois groupes témoins dans la même région et des valeurs normales mesurées dans nos pays. La plombémie d'un adolescent y est voisine de 20 µg/100 ml et augmente régulièrement avec l'âge pour atteindre 30 à 35 µg/100 ml à 50 ans. Le pourcentage d'individus, dans le groupe exposé, qui présentent une plombémie supérieure à 40 µg/100 ml est voisin de 35%.

Une telle plombémie n'est pas nécessairement associée à une intoxication caractérisée mais est potentiellement capable de l'induire après un temps plus ou moins long chez des individus dont les mécanismes d'homéostase sont ou sont devenus déficients. Au vu des premiers chiffres recueillis, cette élévation de la plombémie ne se traduit pas par une modification des mortalités spécifiques de la population considérée; le caractère de l'eau par contre semble exercer une influence notable sur la mortalité par maladies cardio-vasculaires.

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DISCUSSION

MOORE (U.K.)

Ces études présentent des résultats très semblables à ceux que nous avons trouvés à Glasgow en Ecosse. Dans nos études nous avons trouvé des corrélations significatives entre le contenu du plomb dans l'eau, la plombémie et l'ALAD érythrocitaire. Par ailleurs nous avons constaté que la solubilité du plomb dans l'eau est en rapport avec la dureté de l'eau mesurée à l'aide du taux de calcium et de magnésium, et avec le pH. Avez-vous des chiffres pour le contenu de plomb dans l'eau? Comment avez vous mesuré la dureté de l'eau, c.à.d. avez-vous mesuré les taux de calcium et de magnésium ainsi que le pH de l'eau? Disposez-vous de mesures biologiques relatives aux effets du plomb, telles que les activités de l'ALAD?

RONDIA (Belgique)

La concentration du plomb dans l'eau bue par le groupe exposé est difficile à préciser: elle dépend de la *longueur* de la tuyauterie en plomb dans la maison, du *temps de séjour* de l'eau potable dans cette tuyauterie, de la *fréquence* des prélèvements d'eau pour les besoins ménagers et de l'utilisation de l'eau (les feuilles de thé ou le marc de café retiennent tout le plomb tandis que des légumes ne le retiennent que très peu, comme les pommes de terre ou, au contraire, en entier comme le chou). La dose est donc difficile à préciser: on peut grossièrement l'évaluer à 1 à 2 mg/jour.

Les caracteristiques de l'eau ont été précisées dans la communication faite au Symposium d'Amsterdam: la dureté est nulle, le CO_2 dissous est faible, la concentration en acides humiques élevée. L'eau d'un groupe témoin (Eupen, barrage de la Vestre) a la même origine mais est durcie et carbonatée artificiellement; la dissolution du plomb à partir de cette eau est nulle et les conséquences sur la plombémie des habitants sont très favorables.

Les premières mesures biologiques autres que la plombémie sont en cours depuis quelques semaines. Elle comportent une vérification, après un an, de la plombémie et la mesure de l'ALA urinaire, de l'ALAD des érythrocites et de la concentration du plomb dans les cheveux. Les premiers résultats obtenus confirment toutes les corrélations classiquement obtenues par d'autres chercheurs sur les intoxications saturnines à très faibles doses.

INCREASED SUSCEPTIBILITY OF FEMALES TO INORGANIC LEAD

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ABSTRACT

Lead (20 µg/kg body weight/day) administered per os as lead acetate for three weeks to human volunteers of both sexes, caused a significant increase of protoporphyrin IX content of the erythrocytes (PPE) in female subjects only. The lead in blood level (PbB) increased in both groups of volunteers. A control group was included in the study. An increased susceptibility of adult females in comparison to adult males is suggested.

Study supported by grant from EEC.

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Introduction

Older literature contains many suggestions for increased susceptibility of women to inorganic lead. However, critical evaluation shows that data on exposure hardly exist. In a recent review Goyer et al (1972) did not even mention the influence of sex as such. But a few of the factors discussed by them may be relevant: low dietary intake of Ca and P may induce higher retention of lead in the body and may enhance the severity of anemia and biochemical changes in lead poisoning (animal experiments). Iron deficiency results in a microcytic anemia; lead poisoning does the same; a synergism between the two conditions has been suspected. Fe-deficiency also increases Pb in bone.

Because women often may tend to be borderline in relative Fe intake and during pregnancy and lactation also in Ca-intake, above mentioned phenomena from animal experiments may suggest mechanisms for increased susceptibility of women in comparison to males. The first signal of this difference between males and females comes from an unexpected finding of Maxfield et al (1972): in lead exposed dogs female dogs showed an earlier increase in δ-aminolevulinic acid excretion than male dogs.

In our experiments we studied the response of PPE in human volunteers (male and female) exposed to a known daily dose of inorganic lead; we also studied ALAD, ALAU, Hb, but these data are not relevant for this discussion. A full report has been offered for publication elsewhere (Stuik 1974).

Material and Methods

The volunteers were healthy female and male students, 18 to 26 years of age: 5 males served as controls, 5 males received 20 μg Pb^{2+}/kg body weight/day and 5 females, which received the same dose. Lead was administered daily for 21 days (weekends included) as leadacetate solution in glycerol, which was pipetted in cellulose capsules in the exact amount (taking into account body weight) to be taken in the evening after dinner; the control group received capsules with glycerol only. We took blood by venapunction twice a week. PbB was measured twice a week, PPE only once. During the second week after exposure the males received Ca-EDTA (320 mg/day); the female subjects received this in the third week after exposure.

Analytical methods

PbB was measured using an atomic absorption spectrofotometer (Perkin and Elmer, Mode 303), equipped with a burner and air-acetylene flame, at 283 nm; blood samples were prepared for aspiration according to Hessel (1968). PPE was determined according to Schwartz and Wikoff (1952), modified by Schlegel (1972). This method is based upon extraction and purification of the protoporphyrin after which the concentration in the HCl-extract is measured spectrofotometrically.

Results

As shown in Fig.1 mean PbB level of the control group remains rather constant at about 17 µg/100 ml blood during the experiment. The exposed male subjects show an increase from 20.6 µg/100 ml blood (preexposure value) till 40.0 µg/100 ml blood at the end of the second week of exposure and this level hardly increases (till 40.9 µg/100 ml) in the last week. PbB in females increases from 12.7 µg/100 ml blood till 30.4 µg/100 ml blood being the highest point to be reached in the first part of the third week.

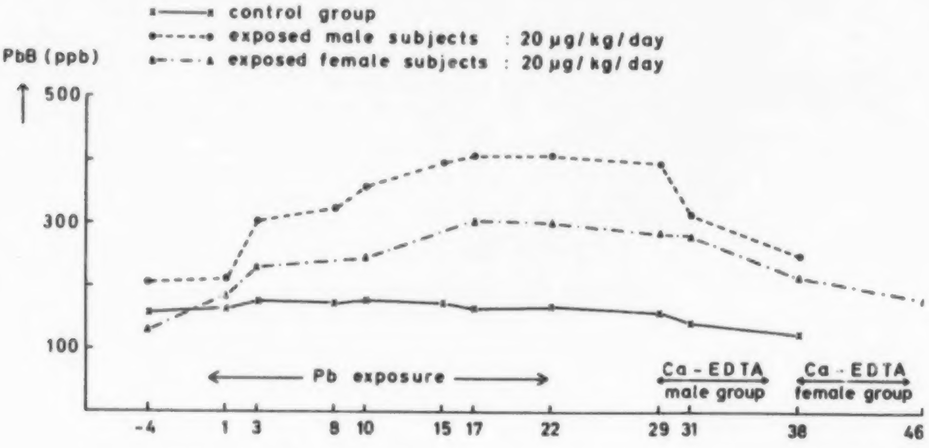

Fig. 1. Average PbB levels, exp. I

PPE does not show a change both in controls and in exposed male group as one can see from Fig.2. The female group however shows a spectacular increase of PPE beginning in the third week and going up till 48.0 µg/100 ml rbc. The increase persists even when PbB is going down again as result of stopping exposure.

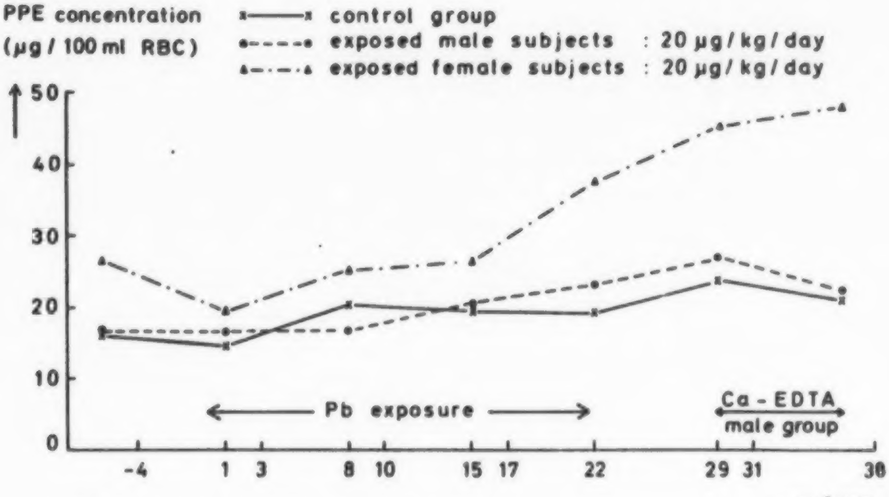

Fig. 2 Average PPE levels, exp. I

Discussion

As we have seen PPE levels in females increased already when PbB level had only increased up to 30 μg/100 ml blood. This was an unexpected finding. Therefore we performed a second experiment with other volunteers and with a female control group; we confirmed the findings of the first study. In that second experiment we also had a male group which ingested 30 μg Pb^{2+}/kg body weight/day. In that male group we saw a slight increase in PPE, however smaller than in females. From both experiments we may draw the conclusion that PPE response in females is earlier and stronger than in males.

It is wellknown that protoporphyrin is formed in maturing erythrocytes in the bone marrow, and that protoporphyrin appears in the peripheral red blood cells with a time lag up to 3 months (Sassa et al, 1973); in our experiments PPE continued to rise in the postexposure period, even with already decreasing PbB. Occurrence of increased PPE levels in relation to PbB therefore preferably should be studied in subjects with a stable PbB, either in long term experiments or in epidemiological studies. Sassa et al (1973) established a coefficient of correlation between PbB and PPE $r = +0.72$ in 138 children mildly exposed to lead; however in 26 children with a stable PbB for at least 3 months this coefficient of correlation rose to r = +0.91.

Because in some regions of the EEG PbB levels in females (and males) have been reported to be in the range of 25-40 µg/100 ml blood, it is strongly advised to design epidemiological studies of PbB and PPE levels in such areas.

It is not possible to explain the underlying mechanisms for the reported difference between adult males and females. One could only give suggestions. Increase of PPE is the result of disturbed utilisation of Fe in the formation of hemoglobin; because women may tend to have a relative Fe-deficiency because of menstrual blood (and Fe) loss, a synergism of Pb exposure and Fe deficiency might be suggested as responsible for the increased response of PPE in females. This suggestion however will have to be tested in experimental and epidemiological studies.

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DISCUSSION

LAUWERYS (Belgium)

I would like to present briefly some data obtained during an epidemiological survey among lead workers, which tend to confirm Professor Zielhuis' observation on volunteers.

Drs Roels, Buchet and myself have compared the red blood cell porphyrin (PE) response and urinary delta-aminolevulinic acid (ALA-U) response in men and women workers chronically exposed to lead. Both groups could be considered as being in a steady state situation with regard to lead exposure.

We have found that for the same degree of lead exposure estimated by lead in blood determination, women exhibit a greater increase in PE than men. ALA-U increase occurs also earlier although the sex difference is less striking for this parameter than for PE. The earlier response in women is probably not due to a relative degree of anaemia by comparison with men, since there is no correlation between hemoglobin (Hb) concentration and PE and thus standardization for the same HB content does not modify the independent effect of lead on the biological responses. Recently we also had the opportunity, in collaboration with the institute of Professor Lafontaine and Dr. Bruaux, to analyse the blood of a group of children living near a lead smelter in Belgium. We found that their PE response to lead resembles that of women or even their PE response may start slightly earlier (i.e. at a lower Pb-B level than in women.

In conclusion, with regard to the heme biosynthesis pathway, women and children seem to be more susceptible than adult men.

ZIELHUIS (Netherlands)

The study of Professor Lauwerys and co-workers in Belgium confirms the experimental data from our study. The study in Belgium was performed after both laboratories had discussed the human volunteer study. The contribution by Professor Lawerys et al. is an excellent example of cooperation between research workers from laboratories who established a good close social and scientific contact.

LYNAM (U.S.A.)

Would you comment on what you believe to be the practical significance of your finding that females exhibit a greater increase and earlier response of PPE than do males for equal blood lead concentrations?

ZIELHUIS (Netherlands)

Our findings are based upon a human volunteer experience; however, they were confirmed in an epidemiological study. This adds to the practical significance of the findings.

In my opinion, the practical consequences for the time being are:

1. More attention should be paid to female response to elevated Pb load. Up to now, occupational health data have been extrapolated to public health, but most data are based upon male subjects. Sex as such appears to be a factor to take into account.

2. The data sound a warning for occupational exposure to females: one may expect evident differences in at least biochemical disturbance of haemosynthesis in females in the range of Pb in blood = 30-70 μg/100 ml, if compared to males. Acceptable exposure limits in industry should be re-evaluated if women are going to be exposed to the same work conditions as males. It is too early to suggest TLV's for females yet. In my opinion women should not be exposed to occupational atmospheric lead. This pertains to non-pregnant women. Very probably potential pregnacy adds another factor for decreasing TLV's for women.

3. In epidemiological studies more attention should be paid to Pb in erythrocytes and the Fe-intake in children and adults.

CLINICAL AND ENVIRONMENTAL CORRELATIONS WITH BLOOD LEAD LEVELS OF CHILDREN IN NEW YORK CITY

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ABSTRACT

During the three year period, 1970-1972, the New York City Health Department-Bureau of Lead Poisoning Control performed over 300,000 blood lead determinations on approximately 265,000 children. This included 23% of all children age 1-6 years in the city (40% of Black and Puerto Rican children).

Parents of children with blood lead levels of 60 microgram per 100ml. or higher were visited at home by a Public Health Nurse. As part of this interview questions were posed concerning any symptoms which the child had in the recent past which could be attributed to the effects of lead.

Data were analyzed by specific symptoms, blood lead level and year of age. The percentage of children with symptoms increased with the increase of blood lead level. However, appro imately 50% of each age group had no symptoms according to the mothers responses at the time of the interview. As might be expected gastrointestinal symptoms were the most common. Seve neurological symptoms such as convulsions and coma were relativ rare.

To analyze the relationship between the blood lead levels young children living in low socioeconomic areas of New York Ci and the degree of lead paint exposure in their housing, indices of housing deterioration were constructed. For each of these indices, a gradient of increasing deterioration with increasing blood lead level was noted.

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This paper will present research findings concerning children one through four years of age who were screened by the lead poisoning control program of New York City during 1971-1972.

Parents of children with blood lead levels of 60+ ug/100ml or higher were asked about the following symptoms: poor appetite, vomiting, pallor. diarrhea, constipation, lethargy and weakness, convulsions, coma, ataxia, irritability, headache, abdominal pain and any other symptoms they might have noticed. The percentage of children reported to have no symptoms is seen in table I. A definite gradient of symptoms in relation to blood lead level is seen. Children at a level of 60 micrograms had the greatest percentage without symptoms. The percentage of asymptomatic children decreased as the lead level increased. Nearly 60% of children with blood lead levels of 60 micrograms were without symptoms, but only about 33% were without symptoms at blood lead levels of 90 micrograms and above.

Table I

Percent of Children Without Symptoms Attributable to Lead
by Blood Lead Level & Age
New York City 1971-72

Lead Level (ug/100ml)	Years of Age			
	1	2	3	4
60	57.0	58.6	59.5	59.9
70	54.1	49.7	55.3	49.1
80	45.5	48.4	46.3	50.0
90	30.0	35.5	31.6	40.0
100+	38.3	31.2	33.3	27.3
Number	835	710	413	296

Each year of age had a similar symptom gradient. However, for each age the symptom complex did vary. Among the major symptoms the incidence of poor appetite, vomiting, lethargy, irritability and abdominal pain increased as the lead level increased. Poor appetite was the most common symptom in all age groups. Reports of abdominal pain increased also by the age of the child with a range of 3% to 10% in children age one and 12% to 36% in children age four years. By its very nature this symptom might be expected to be reported more often in an older child because he is better able to express himself verbally.

Only 37 convulsions were reported for the entire group. Of these, seventeen occurred in children age one year, ten in the two year group, six in the three year group, and four in the four year group. This resulted in an incidence of 2.0%, 1.4%, 1.5% and 1.4% respectively. In the one year age group a 1.0% to 1.5% incidence of convulsions was reported for blood lead levels in the 60-80 microgram range. The incidence rose to 5.0% (2/40) at a level of 90 micrograms and 10.6% (5/47) at a level of 100+ micrograms. Three cases of coma were reported.

Table II

Percent of Children Admitted to Hospital
by Blood Lead Level & Age
New York City 1971-72

Lead Level (ug/100ml)	Years of Age			
	1	2	3	4
60	15.7	19.5	15.2	12.3
70	41.1	43.5	41.6	41.1
80	75.9	81.5	65.1	75.0
90	85.0	97.0	95.0	60.0
100+	98.0	95.9	94.4	83.3
Number	867	745	427	304

The percentage of children admitted to hospital by blood lead level and age is seen in table II. The percentage of children admitted to hospital increased as the blood lead level increased. As can be seen a lead level of 60+ micrograms was not considered to be reason for automatic admission to a hospital. From the data in table II the lead level of the child appears to be a very strong factor in hospital admission, but there was essentially no variation among the different age groups.

In interpreting these findings several factors should be kept in mind: first, the lead level given is the blood sample obtained on first encounter, the level may have been higher before or become higher after this particular sample; two, these symptoms were coexistent with the particular lead level, but were not proven to be caused by an elevated body burden of lead on an individual basis; three, there is a tendency for personnel to look more vigorously for symptoms in children with higher lead levels.

To analyze the relationship between the blood lead levels in young children (1-3 years) living in low socioeconomic areas of New York City and the degree of lead paint exposure in their housing, the homes of three groups of children with different lead levels were chosen for investigation: 1) 10-20 ug/100ml (233 children). 2) 40 ug/100ml (499 children). 3) 60+ ug/100ml (317 children). The results of housing inspections were designated as follows: 1) "No cause for action", i.e. no peeling paint or plaster was found. 2) "Negative", i.e. deteriorated surfaces were found, paint samples were sent to the laboratory for analysis, but no sample was found to contain more than 1.0% lead. 3) "Positive", i.e. one or more paint samples were found to contain more than 1.0% lead.

As seen in table III a gradient of increasing deterioration with increasing blood lead level was apparent. Only 2.7% of homes with children in the 60+ microgram blood lead category were characterized as "no cause for action" in contrast to about 25% of those in the 10-20 microgram group. At the other extreme "positive" apartments were found associated with 27% of black children in the 10-20 microgram group, but 77% of those in the 60+ microgram category.

Table III

Result of Housing Inspection by Blood Lead and Ethnic Group
Among Children (1-3 years)
New York City 1971-1972

Result of inspection	PERCENT					
	Black			Puerto Rican		
	Blood lead (ug/100ml)			Blood lead (ug/100ml)		
	10-20	40	60+	10-20	40	60+
No cause for action	23.4	18.4	2.7	25.4	12.4	2.7
Negative	48.9	26.6	20.2	32.5	22.9	20.7
Positive	27.7	55.0	77.1	42.1	64.7	76.6
Number	47	305	188	114	105	111

Although we have demonstrated a strong correlation between lead in household paint and lead in blood of children the data presented here still show approximately 20% of children with levels of 60 micrograms living in "negative" homes. It should be noted that these data are derived from a single inspection which might best be characterized as thorough but not exhaustive. Our studies of the inspection process itself have indicated that a subsequent inspection would yield at least one new source of leaded paint chips in about half of the "negative" households.

To look at these data from another point of view, indices of housing deterioration were constructed. As seen in table IV, the first index is a ratio in which the numerator is the total number of paint samples taken during inspection and the denominator is the total number of apartments inspected. In the second index the numerator is the total number of paint samples with more than 1% lead ("positive") and the denominator is the number of apartments inspected. The last index is computed as the percent of all samples which were found to be positive. The calculations presented in table IV are based on chemical analysis of 10,187 paint samples obtained from 868 apartments.

Table IV

Indices of Housing Deterioration and Lead Hazard
by Blood Lead and Ethnic Group
Among Children (1-3 years)
New York City 1971-1972

	Black			Puerto Rican		
	Blood lead (ug/100ml)			Blood lead (ug/100ml)		
Index	10-20	40	60+	10-20	40	60+
Total Samples / Apartments Inspected	5.4	8.7	15.1	10.6	13.1	16.8
Positive Samples / Apartments Inspected	0.6	1.9	4.1	1.2	3.0	3.7
Positive Samples / Total Samples	11.9%	21.6%	27.3%	11.0%	23.0%	22.3%

For each of these indices we observe a gradient with higher indices being associated with higher blood lead levels. Children with high blood lead levels lived in apartments where significantly more broken wall surfaces were found and the paint chips obtained were more likely to contain excess lead.

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DISCUSSION

KAMINSKI (U.S.A.)

How many of the children living in what you presented as negative homes in table 1 but with blood lead levels over 60 µg /100 ml were suffering from pica?

GUINEE (U.S.A.)

We did collect that information but we have not tabulated it for this study.

In general we have not stressed a history of pica in the New York program since we find so many children with high blood lead levels with a negative pica history according to the mother We recommended testing all children age 1-6 years, especially age 1-4 years who live in deteriorated housing regardless of pica history.

WILLIAMS (U.K.)

I understood Dr. Guinee to say that the level of blood lead was known to the interviewer who elicited the frequency of symptoms. This would surely introduce a large bias into the apparent increase in frequency of symptoms from a blood lead of 60 µg/100 g. At what level does Dr. Guinee believe that symptoms due to lead begin to occur?

GUINEE (U.S.A)

I mentioned in my presentation that one might expect a tendancy for personnel to look more vigorously for symptoms in children with higher lead levels. We did not find this, but it could affect the data.

Our observers - mostly nurses - found a prevalence of symptoms in the 60 μg and 70 μg/100 ml groups that is about the same as that observed in a similar group of "normal" children. In table I it seems to me that excess symptoms appear above the 80 μg/100ml level.

In studying this situation we have not only the usual variability in susceptibility of individuals but also the problem of discerning which child is irritable due to a lead burden rather than a dozen other couses of irritability.

EPSTEIN (U.S.A.)

Dr. Guinee appears to have overlooked or minimized the role of lead particulates in automobile exhaust as a significant cause of elevated environmental and body burdens of lead. A wide range of studies, including several "roadway" investigations, have demonstrated increased levels of lead in air, street dust and house dust and also their positive associations with elevated blood lead levels. Additionally, it is of interest to note that since New York City recently phased out the use of lead as a gasoline additive there have been significant reductions in lead levels in air and in street dust and also evidence of reduced body burdens of lead in children in various sites in New York City. It is perhaps important to emphasize that such effects may, in part, be attributable to concomitant effective lead paint abatement programs.

GUINEE (U.S.A.)

We have demonstrated strong concomitent variation in the gradient of lead available from deteriorating painted walls and lead in the childs blood, this association supports the thesis that lead paint is a major source of lead available to a child, but not necessarily the sole source. Similar data have not yet presented or reported on lead exposures via air and dust except in populations living under industrial conditions - the smelter towns.

Concerning lead in NYC air - some areas of Queens and Brooklyn with the lowest measured air lead content have the highest incidence of lead poisoning cases whereas the area of upper Manhattan with the highest content of lead in the air has a low case incidence (a child with over 60 μg Pb/100ml of blood is considered a case).

BERLIN (C.E.C.)

In your paper symptoms of different degrees of gravity are lumped together in table I when comparing with blood lead levels.

1. Could you indicate for the various blood levels and ages the percentage of the various symptoms observed?

2. At which blood lead levels below 60 ug/100 ml does the percentage of children with clinical symptoms (and which type of symptoms) become negligible?

GUINEE (U.S.A.)

The symptoms of an increasing lead burden in children are mostly vague. One might best characterise the symptom complex as simply increasing malaise. It is for this reason that the data in table I are presented in terms of percent asymptomatic.

In a control group the percent with these general symptoms and conversly the percent asymptomatic were similar to children with blood lead levels of 60 and 70 ug/100 ml.

This table is, of course, a summary. The expanded data for each symptom, lead level of 60 ug and above, and year of age are available in tables for those wishing to consult them.

MORTALITY IN WORKERS IN LEAD SMELTERS AND LEAD BATTERY PLANTS

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ABSTRACT

The mortality of 7.032 men employed for one or more years in lead production facilities or battery plants was followed over a 23-year period, 1947-70. Lead absorption in many of these men was greatly in excess of currently accepted standards based upon urinary and blood lead concentrations available for a portion of the group. The standardized mortality ratio (SMR) for all causes was 107 for smelter workers and 99 for battery plant workers. Deaths from neoplasms were in slight excess in smelters, but not significantly increased in battery plants. There were no excess deaths from kidney tumors. The SMR for cardiovascular-renal disease was 96 for smelter workers and 101 for battery plant workers, i.e. roughly the same as for the general population, but not as good as would be expected in a population that had been employed. There was definitely no excess in deaths from either stroke or hypertensive heart disease. However, deaths classified as "other hypertensive disease" and "chronic or unspecified nephritis" were higher than expected. The actual numbers of deaths in these last-named categories combined (41 where 19.5 were expected) represented about 3% of all certified deaths. The life expectancy of lead workers was calculated to be approximately the same as that of all U.S. males.

It should be emphasized that there are justifiable objections to the direct application of a study of this type to

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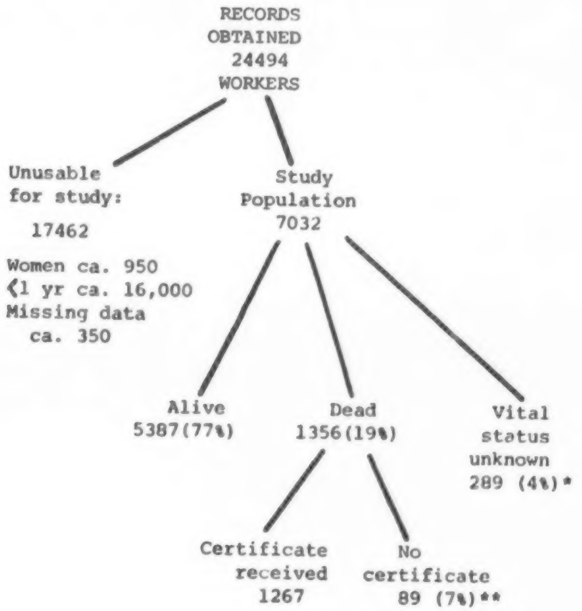
community standard development. Life-shortening illness may be too insensitive an indicator. Factors other than lead, such as arsenic, cadmium and sulfur dioxide were probably involved for some part of the population. There are no children or women represented. The results are therefore more pertinent to workplace standards than to community standards. They support the adequacy of currently recommended biologic standards for the workplace.

Considering the high levels of exposure and the relatively small deviations from expected mortality, one can be optimistic in predicting no detectable impact on mortality for male adults occupationally exposed to lead controlled in conformity to current standars.

Mortality was studied in 7,032 men who had been employed for at least one year in one of 6 lead production facilities ("smelters") or 10 battery plants in the United States during the period January 1, 1946 through December 31, 1970. All plants were in the United States. Selection was based upon the plants' willingness to participate, the availability of records, and geographic distribution. The cohort studied was developed from review of 24,494 personnel records, personally microfilmed by our staff. After elimination of female employees (mainly clerical), men who worked for less than one year, and about 350 with grossly deficient records, a study population of 7,032 men remained. Serial data on urinary and blood lead concentrations were obtained from 9 plants. Although personnel records did not supply information on the race of workers, approximate racial distributions were obtained from questionnaires sent to the participating plants. These indicated that lead production workers were approximately 25% non-white and battery plant workers were 10% non-white, compared with a U.S. proportion of about 11%.

Follow-up to determine vital status was carried out by usual methods, i.e. plant records, telephone, postal service, drivers' license bureaus, retail credit bureaus, and (for all but two plants) by review of records by the Social Security Administration. Follow-up was successful for 96% of the entire population, i.e. 98% of smelter workers and 95% of battery plant workers. If the two plants not included in social security follow-up were omitted, the battery plant follow-up was 99%. The 1356 deaths during the 23 year period from January 1, 1947 through December 31, 1970 were the basis for mortality analyses. Death certificates were obtained for 1,267, or 93%, of the 1,356 known deaths. Figure 1 summarizes establishment of the study population and the subsequent follow-up.

TABLE 1



*193 of these from 2 smaller plants where no Social Security followup
**70 from one plant in Pennsylvania.

Indicators of probable exposures. It was impossible to define the exposures to lead in quantitative terms. The population studied included over 3,000 men who had been working on January 1, 1946, and exposures extended back to 1920 or earlier for some individuals. No quantitative data existed for periods before 1948, and for most plants it began much later.

One indicator of potential exposure is the number of years men had spent in the industry. Table 2 summarizes the employment history of the 7,032 men in the study, based on date of termination or December 31, 1970, whichever came first:

TABLE 2

DISTRIBUTION BY NUMBER OF YEARS EMPLOYED FOR SMELTER AND BATTERY PLANT WORKERS

Years employed*	Per cent of population with more than indicated number of years	
	Smelters	Battery Plants
Over 5	54.0	69.9
10	39.6	53.0
15	31.7	41.1
20	24.0	35.2
25	15.3	29.3
30	9.4	19.2
35	4.7	10.0

*As of date of termination or December 31, 1970, whichever came first.

An indicator of the degree of exposure is information from biologic monitoring. We obtained figures on urinary or blood lead concentrations from nine of the 15 plants which had routine monitoring programs in 1970. (Table 3) Many workers in the study had no recorded levels, because their entire work experience had been before their individual plant began monitoring. PbU values were available for less than 10% of deceased workers; PbB values were available for less than 2%. For this and other reasons it was not possible to attempt correlations between urinary and blood lead values and actual mortality patterns.

TABLE 3

SUMMARY OF URINARY LEAD AND BLOOD LEAD DETERMINATIONS

	Number of analyses	Number of men	Analyses per man (Average)	Per Cent of total (7032)
PbU	97,002	2,274	43	32%
PbB	16,152	1,860	9	26%

Study of the 97,002 urine and the 16,152 blood lead concentrations which were made available, however, provided valuable indirect information as to the relatively high levels of lead absorption by a substantial portion of workers in the plants under study.

Average PbU concentrations were calculated for over 2,000 employees. Plant average means ranged from 125 µg/liter to 187 µg/liter for smelters and from 99 µg/liter to 148 µg/liter for battery plants. Table 4 summarizes the numbers of men whose mean values over a working lifetime exceeded certain indicated values.

Corresponding review of blood lead determinations showed that in 1,850 men, plant average means ranged from 41 to 75 µg/100 g in smelters and from 53 to 67 µg/100 g in battery plants. The numbers of men whose mean values

TABLE 4

NUMBER OF WORKERS WITH 10 OR MORE PbU DETERMINATIONS WHOSE MEAN VALUES EQUALLED OR EXCEEDED INDICATED CONCENTRATIONS (µg/L) (UNCORRECTED)

Type Plant	Numbers of Workers with over 10 determinations	Number with mean values			
		≥150	≥200	≥250	≥300 µg/liter
Smelters	497	289	164	70	27
Battery Plants	1053	249	59	17	7
Total	1550	538	223	87	34

exceeded indicated levels for a working life time were as shown in Table 5.

TABLE 5

NUMBER OF WORKERS WHOSE MEAN PbB VALUES
OVER A WORKING LIFETIME EQUALLED OR EXCEEDED
THE INDICATED CONCENTRATIONS

Type Plant	No. of Workers	Number with mean values ≥40	≥70	≥80	≥100 µg/100 g
Smelters	534	457	138	83	25
Battery plants	1316	976	350	105	52
Totals	1850	1433	488	188	77

Criteria for taking urine and blood samples differed from plant to plant, so the proportions with elevated values cannot be used as an indicator of percentages prevailing in the entire workforce. The absolute number of men with lifetime average values exceeding various important thresholds is striking, however.

Proportionate Mortality. The distribution of certified deaths for 12 selected causes classified by the 1955 revision of the ICD, compared with percentages among U.S. males for 1959 is shown in Table 6.

Proportionate mortality does not take into account the age of the population at risk of dying and the periods in time when each age group was at risk. It can thus be misleading, but is of interest for comparison with other analyses of mortality. It will be noted that there are suggestive excess percentages for malignant neoplasms, other hypertensive diseases, and chronic and unspecified nephritis.

Standardized mortality ratios. The number of deaths observed in the study group was compared with the number which would have been expected in a comparable group in the general male population of the United States, based on person-years of risk in successive time periods (Table 7). The causes of death were classified by applying the rules of the 1955 revision of the International Classification of Disease, Injuries and Causes of Death.

TABLE 6
PERCENTAGES OF 1267 CERTIFIED DEATHS IN SMELTER AND BATTERY PLANT WORKERS, DUE TO SELECTED CAUSES, COMPARED WITH PERCENTAGES AMONG U.S. MALES FOR 1959 (PROPORTIONATE MORTALITY)

Cause of Death	U.S. Males	Smelter Workers	Batteryplant Workers
Major cardiovascular-renal	53.9	45.7	57.3
Vascular lesions of CNS	9.7	7.4	7.1
Hypertensive heart disease	3.2	3.1	3.1
Other hypertensive disease	0.7	2.2	1.4
Chronic & unspecified nephritis	0.7	2.2	1.5
Malignant neoplasms	14.6	21.3	19.7
Of respiratory tract	3.4	6.8	6.5
Of urinary organs	0.9	1.5	0.5
Cirrhosis of liver	1.3	0.6	2.8
Suicide	1.5	4.0	1.7
Motor vehicle accidents	3.0	4.6	1.9
Other	25.7	23.8	16.6

TABLE 7
EXPECTED AND OBSERVED DEATHS BY CAUSE
Jan. 1, 1947 - Dec. 31, 1970
FOR LEAD SMELTER AND BATTERY PLANT WORKERS

Cause of death	Smelters			Battery Plants		
	Obs	Exp	SMR*	Obs	Exp	SMR**
Tuberculosis, all forms	1	4.55	23	5	14.84	37
Bacterial and viral diseases plus influenza and pneumonia	8	8.17	103	23	26.82	92
Malignant neoplasms	69	54.95	133	186	180.34	111
Major cardiovascular renal disease	151	165.29	96	540	575.21	101
Violent deaths	59	40.52	154	62	89.63	74
All other certified causes	36	46.15	82	127	141.45	97
Total for certified causes	32[illegible]	319.64	107	943	1028.37	99
All causes included uncertified	342	319.64		1014	1028.37	
Number of workers		2352			4680	
Person-years		33482			69828	

*Correction of +5.55% applied for 18 missing death certificates

**Correction of +7.52% applied for 71 missing death certificates

The total mortality was approximately the same as that of the general population, 1356 deaths having occurred where 1348 were expected, giving a standardized mortality ratio (SMR) of 101. That for smelter workers was 107 and that for battery plant workers 99. This is higher than for most employed populations (Enterline, 1964 and Sterling, 1964) but does not indicate a serious over-riding hazard to life.

SMR's were calculated for specific causes of death, with the following results (after correction for missing death certificates).

Bacterial and viral diseases, plus influenza and pneumonia. The SMR for smelter workers was 103 and that for battery plant workers was 92, not supporting any increased risk of death from infectious diseases in either group. This grouping was of interest because of concern over a possible effect of lead on immune mechanisms.

Malignant neoplasms. The SMR for "all malignant neoplasms" in smelter workers was 133, slightly elevated and barely significant at the 5% confidence level. As shown in table 8, this was largely explained by a slight excess in tumors of the digestive organs and respiratory system.

TABLE 8 EXPECTED AND OBSERVED DEATHS FOR MALIGNANT NEOPLASMS
Jan. 1, 1947 - Dec. 31, 1970
Lead Smelter and Battery Plant Workers

Cause of death	Smelters			Battery Plant		
	Obs	Exp	SMR*	Obs	Exp	SMR**
All malignant neoplasms(140-205)	69	54.95	133	186	180.34	111
Buccal cavity & pharynx(140-148)	0	1.89	--	6	6.02	107
Digestive organs, peritoneum(150-159)	25	17.63	150	70	61.48	123
Respiratory system(160-164)	22	15.76	148	61	49.51	132
Genital organs(170-179)	4	4.15	101	8	18.57	46
Urinary organs(180-181)	5	2.95	179	5	10.33	52
Leukemia(204)	2	2.40	88	6	7.30	88
Lymphosarcoma, lymphatic and hematopoietic (200-203, 205)	3	3.46	92	7	9.74	77
Other sites	8	6.71	126	23	17.39	142

*Correction of +5.55% applied for 18 missing death certificates

**Correction of +7.52% applied for 71 missing death certificates

Battery plant workers, with an SMR of 111, did not exhibit a significantly high incidence of deaths from malignancy. Since some smelter workers had exposures to arsenic, and analyses based on duration of employment and job descriptions showed no correlations with SMR's for malignant neoplasms, one is not warranted in postulating an association with lead. The 10 tumors of the urinary tract included 6 primary bladder tumors, 1 urethral tumor and 3 kidney tumors. The absence of an excess of renal tumors was of special interest in view of reports of the experimental production of such tumors in rats (Zollinger, 1953) and in mice.

Major cardiovascular-renal disease. Smelter workers exhibited an SMR of 96 for this group of diseases; battery workers had an SMR of 101. Thus the incidence is like that of the general population, and is somewhat higher than that of most employed populations. SMR's for more specific causes are shown in table 9. There was no excess of deaths in either group for "vascular lesions affecting the CNS", i.e. "stroke", the SMR for smelter workers being 109 and that for battery plant workers 82. Dingwall-Fordyce and Lane (1963 had evidence to support an association between prolonged and excessive exposure to lead and death from cerebrovascular accidents, but Malcolm in 1971 reported that reduction of lead exposures was apparently eliminating excessive mortality from this cause. Hypertensive heart disease similarly was not in significant excess in our study, consistent with clinical studies which have failed to show an undue amount of arterial hypertension in lead workers (Belknap, 1936; Cramer and Dahlberg, 1963). However, both smelter workers and battery plant workers had elevated SMR's in the categories of "other hypertensive disease" (389 and 223 respectively), and for chronic and unspecified nephritis (264 and 175 respectively). Actual diagnoses in these two groups were essentially the same; 17 of 20 diagnoses of "other hypertensive disease" included mention of nephroscleriosis and/or uremia. Although the total numbers of deaths were small (41 in all), these deviations are consistent with many reports associating heavy lead exposures with kidney disease (e.g. Goyer and Rhyne, 1973).

Life expectancy. A calculation of the life expectancy of lead workers was made (Table 10), based on the method of Chin Long Chiang (1961). The calculation answered the question. "If a man alive at age 20 is subjected, at each subsequent period of his life, to the same mortality as observed among lead workers in this study, at what age can he expect to

TABLE 9

EXPECTED AND OBSERVED DEATHS FOR MAJOR CARDIOVASCULAR AND RENAL DISEASE Jan. 1, 1947 - Dec. 31, 1970 FOR LEAD SMELTER AND BATTERY PLANT WORKERS

Cause of death with ICD No.	Smelters			Battery Plants		
	Obs	Exp	SMR*	Obs	Exp	SMR**
Major cardiovascular and renal disease (330-334, 400-468, 592-594)	151	165.29	96	540	575.21	101
Vascular lesions affecting CNS(330-34)	24	23.23	109	67	87.93	82
Arteriosclerotic heart disease(420)	77	104.95	77	340	355.70	103
Hypertensive heart disease(440-443)	10	8.72	121	29	32.30	97
Other hypertensive disease(444-447)	7	1.90	389	13	6.26	223
Chronic & unspecified nephritis(592-594)	7	2.80	264	14	8.58	175
Rheumatic heart disease; endocarditis	12	11.12	114	42	37.80	119
Other	14	12.57	118	35	46.64	81

*Correction of +5.55% applied for 18 missing death certificates

**Correction of +7.52% applied for 71 missing death certificates

die?" The calculated figure for smelter workers was 69.7 years, and that for battery plant workers was 71.5 years. The corresponding figures for U. S. males in 1949-51 was 68.9 years, that for 1959-61 was 69.8 years.

TABLE 10

EXPECTED AGE AT DEATH

AFTER AGE 20

Smelter workers	69.7*
Battery plant workers	71.5*
U.S. males 1949-51	68.9
U.S. males 1959-61	69.8
U.S. white males 1969	70.1
U.S. non-white males 1969	63.9
(Steel workers	72.6)**

*Method of Chiang, Biometrics 17:57, 1961

**Calculated from data of Lloyd & Ciocco.

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DISCUSSION

LAUWERYS (Belgium)

You have found an excessive number of deaths by urinary tract cancer in smelter workers but not in battery plant workers. The smelter workers are also probably exposed to cadmium. It has been suggested that Cd could be responsible for prostrate cancer in man but this remains an hypothesis.

I wonder whether it would not be possible to look at the Cd exposure of the smelter workers as a possible etiologie factor of the increase SMR for urinary tract cancer in this group?

COOPER (U.S.A.)

Although the SMR for urinary tract cancer in smelter workers appeared high the actual number (5) was too small for application of a test of significance. The diagnoses were mainly tumors of the urinary bladder. Prostatic tumors were coded under genital tract tumors and were not in excess. I do not think, from the information available to us and the relatively few individuals who had high cadmium exposures, that we can glean much from our data relative to the hypothesis you wish to test.

HERNBERG (Finland)

It is generally known that the total mortality of any occupational group is likely to be considerably lower than that of the age-and-sex standardized general population, because of selection and other related factors.

Thus, your data should ideally be compared to those of one, or preferably, several comparison cohorts. I agree that this is not always easy to accomplish: in such a case one must use data from the general population. But I would like to stress that, in this case, the comparison yields an underestimation of the actual total mortality - your finding of no excess mortality in fact denotes that there might exist an excess mortality as compared to suitable comparison cohorts, i.e. other occupationally active groups. Your findings on the case-specific mortality support such a conclusion.

COOPER (U.S.A.)

In my brief oral presentation, I apparently did not sufficiently emphasize the important and valid point you have made. This was covered in the full report. There is no entirely satisfactory population of employed persons available for comparison. A number of recent occupational cohort studies have shown that SMR's of 80 to 90 are common, presumably because of selection out of the work force of many individuals with life-shortening conditions and possibly because of other favorable factors associated with work.

EPSTEIN (U.S.A.)

It is of interest to note that approximately 25% of the workers studied had blood lead levels for unspecified periods in excess of 80 µg/100 g. This clearly represents minimal confirmatory evidence of excessive environmental lead burdens in workers in smelting and related industries.

The reported studies appear to reflect overall mortality rather than morbidity. They thus cannot possibly reflect a wide range of disease syndromes and ill health from chronic lead poisoning unless these terminated in premature death. These data cannot be used in any way to support the contention that chronic lead poisoning is not a major cause of occupational morbidity. Additionally, the data indicate an apparently significant excess of mortality from hypertensive renal disease.

COOPER (U.S.A.)

The average urine and blood levels cover periods dating back to 1950 with the majority having come from after 1960. They do not relate directly to the study group, most of whom worked before biologic monitoring was begun. They are only an indirect index which I agree shows excessive absorption of lead. This may have been even higher in the earlier history of some of these men and was certainly not lower. With such high levels of exposure, which are certainly not acceptable levels, we think it is of interest that there was no stronger impact on mortality. The limitations on extrapolation of this study, including the absence of women and children, were included in the oral presentation.

One final comment - the percentages of men with high urinary and blood lead averages did not represent percentages of the entire work force but rather percentages of men who had samples taken. The complete report also contains such evidence as we have on changes, as we proceed from 1950 to 1974.

EVALUATION OF LONG-TERM EFFECTS OF ELEVATED BLOOD LEAD CONCENTRATIONS IN ASYMPTOMATIC CHILDREN

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ABSTRACT

The purpose of this paper is to contribute objective data toward the often expressed hypothesis that subclinical elevation of body lead burden may have permanent deleterious effects on the physical and mental development of children.

A unique group of children discovered accidentally presented a naturally occurring experimental model for the study.

This group of experimental children with good nutrition, no anemia, and happy homes in a three generation small community, leave elevated blood lead as an apparent isolated significant variable.

The children were matched with a control group, tested on a blind basis and the results analyzed for any significant differences.

Results of the study are presented as objective evidence to aid in determining safe levels of lead exposure. In this group of children prolonged exposure to subclinical lead levels in the 40-80 µg/100 ml range has not resulted in apparent deleterious effects.

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1. Introduction

In January 1972, a unique opportunity to test the effects of prolonged subclinical elevations of lead in children was discovered in El Paso, Texas.

Smeltertown, isolated between the Rio Grand river and a large smelter, had existed for some 80 years in an arid climate receiving approximately 6 inches of rain per year. There was no drainage system, no grass, dirt streets, dirt yards, and a few trees.

Available high content lead paint in the area was minimal and similar to El Paso. Soil lead samples averaged 3300 parts per million. Of 206 children in the area, 129 had elevations over 40 mcg% which is an incidence of 67%. Four other studies in El Paso tested 836 children with only 14 over 40 mcg%--an incidence of 1.7% (Table I). These were healthy, active, and happy children whose source of lead was presumed to be inhalation and ingestion of contaminated soil during the course of their normal year round outdoor play and activity.

2. Clinical Model for Study

2.1 Test Group

One hundred thirty-eight children agreed to participate in the study. Thirty-seven had blood lead levels less than 40 mcg%, 26 were 40-49 mcg%, 23 were 50-59 mcg%, and 43 were over 60 mcg% (Table II). They were rather evenly distributed in age from 21 months to 18 years of age with a median of 9 years. (Table III) Sex distribution was 68 male and 70 female.

2.2 Controls

Control groups were chosen from El Paso children residing outside of Smeltertown and matched with the lead children by age,

TABLE I.

LEAD SCREENING RESULTS

Number of children	Total	Blood Lead Values <40 mcg%	>40 mcg%	% >40 mcg%
SMELTERTOWN				
Study Group	138	37	101	73%
NonStudy Group with known blood lead	55	27	28	51%
without known blood lead	13			
subtotal	68			
TOTAL	206	64	129	67%
EL PASO outside Smeltertown				
Initial tests by smelter	75	75	0	0
CDC and BCEM screen	550	538	12	2%
Mesita School children	69	69	0	0
ILZRO study controls	142	140	2	1.4%
TOTAL	836	822	14	1.7%
OLD FT. BLISS	144	128	16	11%

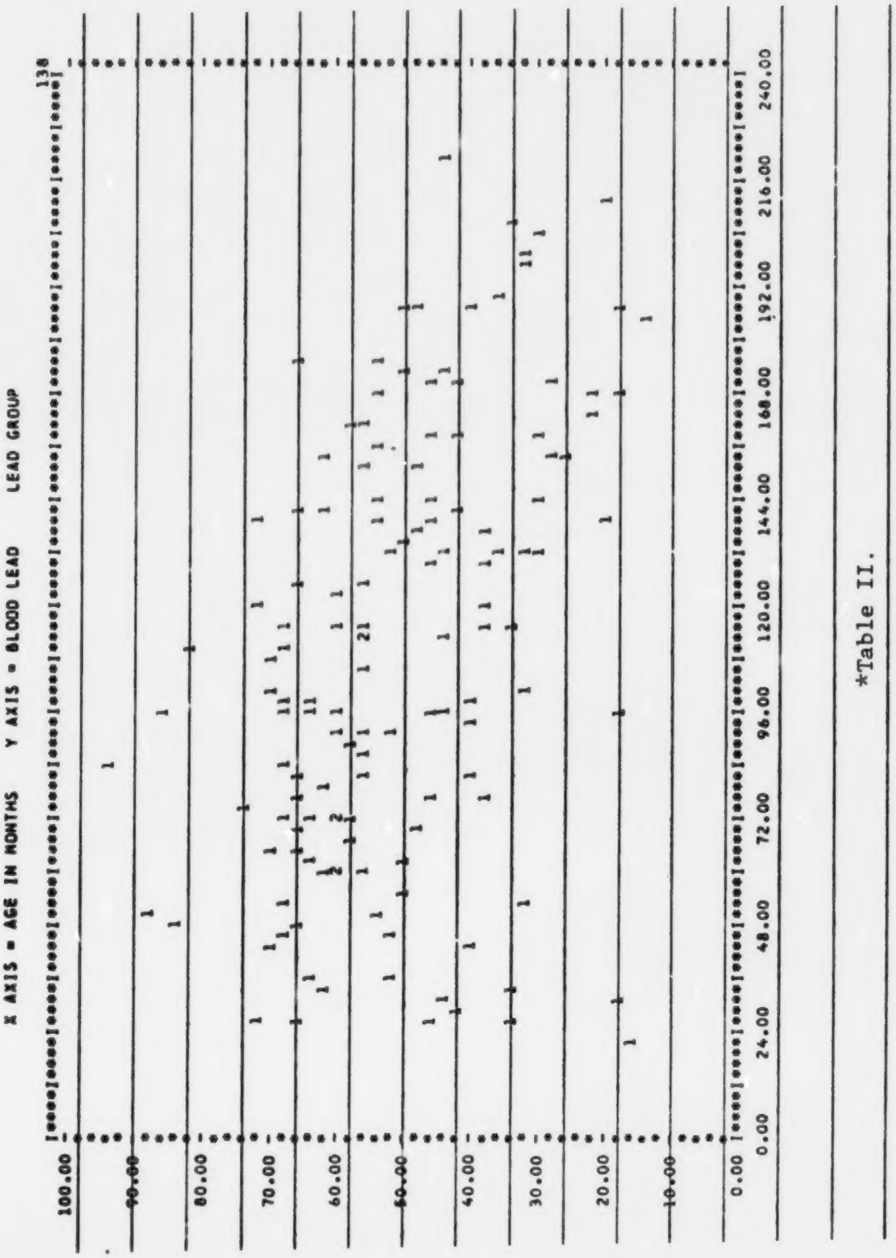
X AXIS = AGE IN MONTHS   Y AXIS = BLOOD LEAD   LEAD GROUP
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
24.00
48.00
72.00
96.00
120.00
144.00
168.00
192.00
216.00
240.00
138

*Table II.

Table III.

CONTROL MATCHING DATA

	Lead	City Control	Rural
Average Age Difference	- .69 day	+ .69 day	
Lead Under 8 Years	+4.64 day	----	-4.64
RACE			
% Mexican/American			
Father	94%	96%	95%
Mother	97%	99%	93%
Child	96%	97%	96%
JOB LEVEL			
Father	1.53	1.43	1.70
Mother	1.06	1.10	1.07
EDUCATION			
Father	7.54 years	7.83 years	8.45 years
Mother	7.27 years	6.77 years	7.65 years
INCOME (Annual)			
Group Average	2.27	1.92	2.42
Dollar Approx. of Annual Income	4,540	3,840	4,840

sex, race, and family income. A duplicate control group for children under 8 years of age was obtained from a rural community approximately 12 miles from the smelter.

2.3 Matching

Spanish is the principal language of all groups. Similarity between the controls and the lead children can be seen on the tabulation of their matching data in Table IV.

3. Procedure

All children were evaluated on a blind basis by examiners who were given only a randomly selected study number, first name, and age for each child. Each child had a history questionnaire answered by the parents, a physical and neurological examination, nerve conduction test, laboratory studies, X-rays, and a psychometric evaluation using tests appropriate for the age.

4. Analysis

The lead exposed children were compared to their matched control as a total group, and subgroups based on blood levels previously mentioned. An additional attempt was made to seek out the high risk children by combining with the blood levels four other parameters of lead exposure. The free erythrocyte protoporphryin level, living in the area during the critical development of birth to three years of age, a minimum of at least two years living in the area, and positive X-ray findings of lead exposure. Forty-four children with 4 out of these 5 parameters positive were placed in a Serious group and when combined with 31 children having 3 of the 5 parameters positive made up a so-called Moderate group of 75 children with at least 3 of the 5 parameterspositive. Statistical significance refers to a level of 0.05.

TABLE IV.

CONTROL MATCHING DATA

	Lead	City Control	Rural
Average Age Difference between Test Child and Control Child	- .69 day	+. 69 day	
Lead Children under 8 years and Rural Control Child.	+4.64 day	- - -	-4.64 day
RACE			
% Mexican/American			
Father	94%	96%	95%
Mother	97%	99%	93%
Child	96%	97%	96%
JOB LEVEL			
Father	1.53	1.43	1.70
Mother	1.06	1.10	1.07
EDUCATION			
Father	7.54 years	7.83 years	8.45 years
Mother	7.27 years	6.77 years	7.65 years
INCOME (annual)			
Group Average	2.27	1.92	2.42
Dollar Approximation of Income	4,540	3,840	4,840

*Scale--Common Labor = 1, Skilled Labor = 2
Service Occupation = 3, Professional = 4

* Scale--Annual Income

<3,000 = 1
3,000-5,000 = 2
5,000-7,000 = 3
7,000-10,000 = 4
>10,000 = 5

TABLE V.

EXPECTED SIGNIFICANT DIFFERENCES

	Lead		Control		Rural	
Time in Area	**No.**	**Years**	**No.**	**Years**	**No.**	**Years**
FATHER	112	22	7	7½	2	9½
MOTHER	135	23	8	8	0	0
CHILD	138	7	0	0	0	0
Blood Lead Level	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Total Group	M-50	(14-93)	M-20	(7-43)	M-16	(10-28)
Moderate Group	M-61	(14-93)	M-22	(8-40)		
Children< 8 Year Age	M-56	(15-93)	M-23	(10-43)	M-16	(10-28)
Free Erythrocyte Protoprophryin						
Total Group	M-186	(20-710)	M-80	(26-303)	M-67	(21-167)
Moderate Group	M-239	(68-710)	M-84	(26-303)		
Children< 8 Year Age	M-195	(39-550)	M-91	(26-303)	M-67	(21-167)

5. Results

There were the expected significant differences between the lead children and their controls in terms of time in the area, blood lead values, and protoporphryin levels. (Table V)

There were no significant differences in the birth history conditions, complications of delivery, neonatal course or birth weight.

These were essentially healthy noncomplaining children.

Fourteen non-specific complaints frequently listed as suggestive of lead poisoning, but not normally seen at this level, were graded on a rating of 1-4 severity in each of the complaints giving a range of 14 to 56 for the group. Their mean scores were 15.27 for the total leads, 14.51 for the city controls, and 14.83 for the rural controls.

Past history on the basis of serious illness and residual effects were rated on a scale of 2-8. Resulting means were 2.46 for lead, 2.47 for control, and 2.42 for rural controls.

Mean heights and weights for all groups were almost identical indicating close matching, good nutrition, and normal growth for age.

The general physical examination and neurological examination was normal. Finger-to-thumb opposition and rapid alternating hand movements showed expected variance with age, but no significant difference with controls.

Hemoglobin and hematocrit values were normal. Group mean average hemoglobin ranged from 14.3 grams in lead children less than eight years of age to 16.8 grams in total controls--adjusted for age to percentage of normal, the range was 98.1% to 102.2%. Eight of nine lead groups were a shade lower than their contrels, but none

Table VI.
COMPARISON OF I.Q. TESTS

		TOTAL LEAD GROUP					MODERATE LEAD GROUP					LEAD< 8 YR.& RURAL					RURAL & CITY CONTROL				
		Pb.	Con	No.	F	T	Mod.	Con.	No.	F	T	Pb<8	Con.	No.	F	T	Rural	City	No.	F	T
WISC	Verbal I.Q.	86.9	86.9	99	1.15	0.04	87.0	88.1	52	1.09	0.50	93.6	89.4	18	1.55	0.93	89.4	92.3	17	2.66	0.66
	Performance	95.2	88.9	99	1.30	1.26	94.9	95.8	52	1.02	0.37	100.6	97.5	18	1.55	0.83	97.5	95.3	17	1.23	0.55
	Full Scale	90.1	88.9	99	1.04	0.73	90.0	91.0	52	1.16	0.39	96.7	92.5	18	1.59	1.01	92.5	93.1	17	3.26	0.14
McCARTHY	Verbal	41.6	42.1	29	1.21	0.20	39.5	40.5	20	1.26	0.36	42.0	39.4	22	1.14	0.90	39.4	40.7	23	1.14	0.49
	Perceptual	42.1	41.0	29	1.78	0.52	41.8	41.1	20	1.79	0.22	42.3	39.6	22	2.19	1.04	39.6	39.3	23	1.01	0.11
	Quantitative	39.5	42.0	29	1.22	0.99	39.4	39.1	20	1.17	0.10	39.6	39.0	22	1.47	0.21	39.0	41.2	23	1.72	0.76
	General Cognitive	83.2	84.4	29	1.09	0.32	80.9	82.0	20	1.22	0.26	83.8	80.2	22	1.01	0.81	80.2	81.9	23	1.13	0.39
	Peabody	83.8	86.9	115	1.07	1.44	83.1	85.9	60	1.07	0.86	83.0	84.6	27	1.22	0.32	84.6	84.0	17	1.39	0.12
	Draw-A-Person	87.5	87.3	109	1.16	0.07	91.4	89.4	58	1.16	0.65	95.4	91.3	23	1.72	0.73	91.3	90.6	17	1.39	0.12
WRAT	Reading Standard Score	88.7	90.0	111	1.60	0.65	89.9	87.6	57	1.85	0.98	89.3	89.2	23	1.72	0.05	89.2	87.7	17	1.02	0.49
	Arithmetic Standard Score	86.1	86.6	111	1.16	0.26	88.7	87.6	57	1.18	0.50	88.3	90.0	23	1.33	0.34	90.0	90.0	17	1.16	0.01
	Spelling Standard Score	87.3	87.0	111	1.16	0.16	89.4	85.7	57	1.14	1.69	95.0	91.6	23	1.14	0.98	91.6	89.6	17	1.55	0.49

were significantly different.

Nerve conduction test in 108 lead children and 89 control children failed to show any significant difference between the leads and controls.

Hyperactivity as a possible manifestation of subtle lead toxicity was evaluated by physician examination, history from parents, and school teacher evaluation. Test values were actually a shade in favor of lead children, but no statistical difference could be shown.

Children under six years of age were tested by the McCarthy Scales of Children's Abilities giving a general cognitive scaled score of 83.24 for lead against 84.43 for the control group. None of the scaled scored values were significant.

The Berry Developmental Test of Motor Integration also failed to show any significant differences in the children under six years of age.

For the children over 6 years of age, the Peabody Picture Vocabulary Test slightly favored the controls but the Draw-A-Person Test favored the lead group, neither difference was significant.

A Weschler Intelligence Scale for Children gave a verbal I.Q. of 86.86 for lead against 86.94 for controls; performance I.Q. of 95.19 for lead against 93.06 for control and a total I.Q. of 90.95 for lead against 88.89 for control. These and all subgroups showed no significant differences. Table VI.

No significant differences occurred in the Wide Range Achievement Test, in the Wepman Auditory Discrimination Test, in the Bender Gestalt with Koppitz scoring test, and the Oseretsky Test of Motor Development.

A Frostig Test of Eye and Motor Function showed one isolated lead advantage over the rural group, and in three categories in the moderate lead group the male had an advantage over the females.

There were some significant differences in the California Test of Personality which showed up in the total group and almost disappeared in the moderate group. In the group less than 40, there were more differences than in the group greater than 60. We believe these differences are not lead related but are related to their historical geographic isolation, closing of their school, adverse news media exposure, and forced displacement from their homesteads.

6. Conclusions

The primary problem of lead poisoning in children remains the ingestion of high content lead paint by economically and socially deprived anaemic toddlers. Any source of lead causing undue absorption in humans which might be added to the problem must be controlled. From a practical standpoint, the problem with Smeltertown has been eliminated. The village is gone. Former residents have been scattered throughout El Paso, their rent has gone up and their money for food has gone down. Most of them would move back to Smeltertown if they could. The children of Smeltertown will be remembered, however, for the contribution they have made to our knowledge of lead in humans by demonstrating that children who are healthy, well nourished, and not anaemic may carry significant elevations of the blood lead levels in the range of 40 mcg% to 80 mcg% over a period of many years without apparent deleterious effects.

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DISCUSSION

NEEDLEMAN (U.S.A.)

I am puzzled by the discrepancy between your data, and those reported in the May 4, 1974 Bulletin of the Center of Disease Control. That report showed a significant deficit in performance IQ of children from Smeltertown USA whose blood lead levels were greater than 40 ug/100 ml. Did your report deal with the same sample? If so, please explain the difference in your findings?

My second question is of a sociological nature and grows our of your concluding comments which I take to be sociological. You indicate that the families who once lived in Smeltertown are now dispersed, and their situation worsened, and that this grew out of excessive publicity. This seems to imply that people near point sources of pollutants have only two choices: to bear the exposure or to leave. On reflection, do you not feel that other options could exist? Perhaps environmental control is one.

McNEIL (U.S.A.)

1. Your question concerning the study reported in CDC morbidity mortality weekly report (MMR) is indeed appropriate. Lack of data in the report make analysis difficult but several things seem apparent.

a) The primary separation of test and control groups is not based on residence in Smeltertown. I think this is a basic requirment to justify the probability of prolonged abnormal lead exposure. Their groups are mixed with 24 in lead group and 36 in control group from Smeltertown. The remaining 26 in lead exposed group are included on basis of initial blood lead level. 48% of the total lead group was below 40 ug% on second blood lead analysis.

b) The average age differential of 1.2 years between lead and control children (ours was .69 days) is a very critical factor to performance in these developing years.

c) The WPPSI test used for 3 and 4 year old children in the study reported in MMR was not normed on Spanish sur-named children. We eliminated the WPPSI from our originally programmed study on the recommendation of Dr. Ptasink and our steering committee who considered it a poor testing instrument for this age in our Latin American culture. When combined with the age differential the weakness of this test becomes even more critical.

d) They apparently averaged the results of their WPPSI test and their WISC test. These are two different tests with different norms and averaging of the data is not proper.

e) The performance section of the weckster test was designed to measure non verbal cognitive functioning, that is, ability to reason without the need to make verbal response. It is not a measure of fine motor function.

f) Poorly matched control group is probably a significant factor but for the present we must limit our comments to the published report.

g) The only other abnormal finding reported was in the Balch tapping test which we believe was designed by one of the co-authors. No breakdown in terms of age performance is given. I do not know if standards for age have been determined. This is a measure of performance that should improve with increasing age.

I do not think the two studies are comparable in terms of control matching groups tested, number and appropriateness of tests used, or in the method of testing.

I do not think the isolated difference in performance I.Q. and the Balch tapping test with all other modalities being similar in their two different mixed groups is sufficient evidence to support the conclusions they have drawn.

2. The people of Smeltertown were the subjects of national adverse publicity which I think may be a factor in the results of the California test of personality. The publicity is not responsible for the primary problem. They were given priority for federally subsidized housing. Their assigned rent on basis of income still represented a rent increase for many families of 2 to 3 times what they had been paying. In this way their situation was worse.

The primary fule for treatment of children with excessive lead adsorbtion is to eliminate the source of lead. The persistent suggestion that these levels may be harmful could not be ignored. Elimination of the area may be environmental control in the extreme but in this situation the most practical and safest method.

GOLDSMITH (U.S.A.)

Several aspects of your paper require comment. I shall address two:

Your sample of 106 out of 238 (numbers by recollection) is a small proportion to provide a basis for generalization. How do you account for this level of participation. Were your subjects paid (or were their parents)?

You report on the tests for differences in hemoglobin in eight out of nine test groups (in which a "shade" of difference was found). By a sign test statistic, this is evidence of a statistically significant anemia in lead exposed subjects. How then can you allege that these had "no deleterious" effect?

Kindly provide the data on hemoglobin levels in these groups in your reply.

McNEIL (U.S.A.)

1. The correct number of children was 138 out of 206 total population. Ideally we wanted the entire population to be in the study. I think 67% is an exceptionally good response. In an effort to get the cooperation of all parties concerned we offered the parents of all the children the opportunity to sign the permit form. This form stated in detail who was doing the study, who was finding the study, what tests were to be done and the purpose for doing the study. There was NO money involved. They were told that they could have the results of the tests on their own children.

I think a significant factor was the lack of inconvience for the parents. The children were picked up for their exams and returned home by study personnel so that parents did not have to miss work or obtain baby sitters. Children were rewarded for their cooperation because of the time involved with lollipops, cool aid, cookies and one trip to McDonals's hamburgers. This was done to discourage children from dropping out of the study prior to completion of tests. There was no selection by the investigators, we wanted all of them.

Examination of known parameters in the non study group indicate similar age, time in area, lack of physical complaints, or history of past illness. 55 of the group had known lead values with a mean of 43.49 ug%.

2. In terms of anemia I do not think we are using the word deleterious in the same context. We were looking for any permanent deleterious effects rather than reversible transitory deleterious effects. With our blood lead and F.E.P. values we expected more anemia than was shown. Increased lead burden may result in anemia but in this group of children they have apparently compensated for the effect to maintain normal levels. Although slightly lower than the controls the differences are not statistically significant.

The summary of data on haemoglobin levels in these groups are provided below.

HEMOGLOBIN AND HEMATOCRIT

GROUP	HIGH		LOW		MEAN		% NORMAL	
	Gm.%	Mm.	Gm.%	Mm.	Gm.%	Mm	Adjusted for age	
Total Lead	16.2	47	10.3	30	13.56	38.45	98.1	93.4
Total Control	16.8	47	10.5	31	13.76	38.86	99.8	94
Serious Lead	15	43	10.8	30	13.16	37.45	98.5	93.9
Control	15.4	43	10.5	31	13.37	38	99.9	95.1
Moderate Lead	16.2	46	10.3	30	13.31	37.80	98.4	93.7
Control	15.8	45	10.5	31	13.49	38.26	99.7	94
Lead < 8 years	14.3	41	10.3	30	12.86	36.4	98.8	93.9
Rural Control	14.4	42	10.0	31	12.95	36.8	99.7	94.8
City Control	15	42	11	31	13.24	37.07	101.8	94.3
Lead < 40	16.1	46	12.4	35	13.89	39.35	98.1	93.1
Control	15.9	45	11	31	13.96	39.37	98.9	93.3
Lead 40-49	16.2	47	11	34	13.84	39.32	97.6	92.9
Control	16.8	46	13	36	14.40	40.44	102.2	96.1
Lead 50-59	15.9	46	11.8	33	13.66	38.1	98.9	93.4
Control	16.2	47	10.5	31	13.46	38	98	91.3
Lead > 60	15	43	10.3	30	13.05	37.44	97.7	93.9
Control	15.4	43	11.5	33	13.45	38.1	100.6	95.3
Lead > 40	16.2	47	10.3	30	13.44	38.12	98.1	93.5
Control	16.8	47	10.5	31	13.69	38.67	100.1	94.2

DAVID (U.S.A.)

1. I understood the speaker to say that the inhabitants of Smeltertown who were studied had a very long family history of living there. Might it not be reasonable to infer from this that they may represent a uniquely based group. That is, those whose resistance to lead toxicity was high, stayed and were studied while those whose resistance was normal or low left Smeltertown, because of illness that they inferred was due to the proximity of the smelter and were not available to study.

2. How many children studied were male between the ages of 5 - 10 (the time span and sex in which hyperactivity occurs)?

McNEIL (U.S.A.)

1. I do not think we have any evidence for such an inference. We could see no apparent difference by history between the families with long residence and those with short residence. We did not find any history suggesting a significant health problem. There were some families that had moved out for various reasons in years passed and then returned to Smeltertown.

2. Breakdown of the children in the age group of 5 to 10 years is:

Lead study group	30 male	32 female
City control group	30 male	32 female
Rural control group	17 male	9 female

I do not agree with the statement that hyperactivity occurs at age of 5 to 10 years. I think this is the age when everyone becomes aware of it because of disturbances at school and other activities away from the home.

CARNOW (U.S.A.)

It may be interesting to the participants to know how the El Paso children were found. Examination of emission data from the smelter by our group in Chicago lead to prediction that children with high lead levels would be found - and indeed when we examined the blood, this was true for virtually all children in Smeltertown less than 8 years of age.

We also carried out a neutral study of more than 173 children - many of the same ones described here as healthy, happy children. They may have improved since 1972 (many were hospitalized and chelated) - but even to the present time we found many with moderate to severe anemias, symptoms of lethargy and excessive sleepiness, classic irritable child syndromes in many, and in significant numbers gross symptoms relating to the gastro intestinal system. Two had convulsive disorders, one had a partial foot drop and in the first examine in detail - gross E E g findings were present in 4, along with learning deficits and other neurophysiologic abnormalities. A second study following ours, by the Communicable Disease Center of the Federal Government apparently found very similar results (personal communication). I cannot understand the discrepancies with this study and would like them explained.

One other matter. `The presenter of this work - adds for reasons I cannot imagine - an expression of great concern at these people being displaced from their homes as though an injustice had been done. In fact thev were moved out into far superior federal housing from what were mostly tinv wooden, dirt floor structures. The reason of course, was that the soil and dust lead levels was as high as 20,000 ppm in some samples - and certainly the children had to be removed from such a large source of a very toxic material.

McNEIL (U.S.A.)

This is a statement and not a direct question but does imply discrepancies which must be discussed.

There is one reported case of lead encephalopathy in El Paso in 1933 by a now decreased pediatrician from the burning of old batteries for fuel. There were 3 hospital admissions in the past 20 vears prior to 1972 at El Paso's two major hospitals for possible lead poisoning. All 3 were adults and the diagnosis was not confirmed.

The city of El Paso in preparation of their suit against the smelter for air pollution did engage Dr. Carnow as a consultant and he did reportedly suggest the blood testing. Five asymptomatic children were initially admitted to the City-County hospital of which I was the outgoing chief of staff.

I have reviewed the hospital records at the City-County hospital in conjunction with the study which I reported. I could not find the abnormalities and symptoms mentioned by Dr. Carnow in the hospital records.

The bulk of the Smeltertown testing was conducted by the smelter. When blood lead elevations were discovered the parents were give the choice of reporting to the City-County hospital or to be seen by their own doctor or to be examined by me.

It is a matter of record in the court proceedings as to the differences in findings as reported by Dr. Carnow and as reported by the other physicians who examined the children. Due to the discrepancies in findings by the various physicians who knew they were examining children with blood lead elevations it seemed obvious that a controlled study with children examined by examiners who did not know their identity would be necessary to resolve the question of possible damage from lead. Most of the limited chelation therapy that was given was in 1972 when the discovery was made. Therapy was given because of laboratory results and not for symptoms. Subsequent developments indicated a more conservative approach to treatment.

In review of the medical progress of these children by the court appointed lead committee it was apparent that the majority of the children were under my care. I then prepared my proposal for the study that has been presented here.

The discrepancies with the study reported in the CDC morbidity mortality weekly report have been covered in my response to Dr. Needleman's question.

Dr. Carnow's impression of Smeltertown is typical of most people who pass through and is similar to the impression I held for my first 17 years in El Paso. Intimate knowledge of the area gave me a different impression. These were not tiny wooden dirt floor structures. They were adobe block, cinder block, or brick structures which they had personnally built or improved. They were proud of their homes.

The new housing is far superior but the number that moved and the time it took them to move indicated a different feeling by the residents.

UNTERSUCHUNGEN ZUM ZUSAMMENHANG ZWISCHEN DEM BLUTBLEISPIEGEL BEI NEUGEBORENEN UND DER BLEIIMMISSIONSBELASTUNG DER MUTTER AM WOHNORT

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KURZFASSUNG

In einem gemeinsamen Untersuchungsprogramm der Kinderklinik, des Hygieneinstituts der Gesamthochschule und der Landesansalt für Immissions- und Bodennutzungsschutz in Essen wurde die Beziehung des Blutbleispiegels von Neugeborenen und ihrer Mütter in Abhängigkeit von der Immissionsbelastung am Wohnort untersucht.

Hierbei wurden folgende Zusammenhänge festgestellt:

1. *Die schon in der Literatur diskutierte Korrelation zwischen kindlichem und mütterlichem Blutbleispiegel wurde bestätigt.*

2. *Ein- und zweieiige Zwillinge hatten fast identische Blutbleiwerte.*

3. *Sowohl der mütterliche als auch der kindliche Blutbleispiegel waren von der Immissionsbelastung am Wohnort abhängig.*

4. *Der Wert eines biologischen Überwachungssystems zur Ermittlung der Bleiimmissionsbelastung konnte aufgrund seiner Beziehung zum kindlichen und mütterlichen Blutbleispiegel aufgezeigt werden.*

ABSTRACT

In a programme of investigations conducted jointly by the Children's Clinic, the University Hygiene Institute and the Land Institute for Pollution Control and Environmental Protection a study was made of the relation between the blood lead levels in new-born babies and their mothers as a function of the degree of pollution at the mothers' domicile.

The following correlations were established:

1. *The correlation between blood lead levels in the mother and the child, which has already been discussed in the literature, was confirmed.*
2. *Monozygotic and bizygotic twins showed almost identical blood lead levels.*
3. *The blood lead levels of both the mother and the child were dependent on the pollution at their domicile.*
4. *The value was demonstrated of a biological monitoring system for the determination of exposure to lead in view of its relationship with the blood lead levels in the child and the mother.*

Einführung

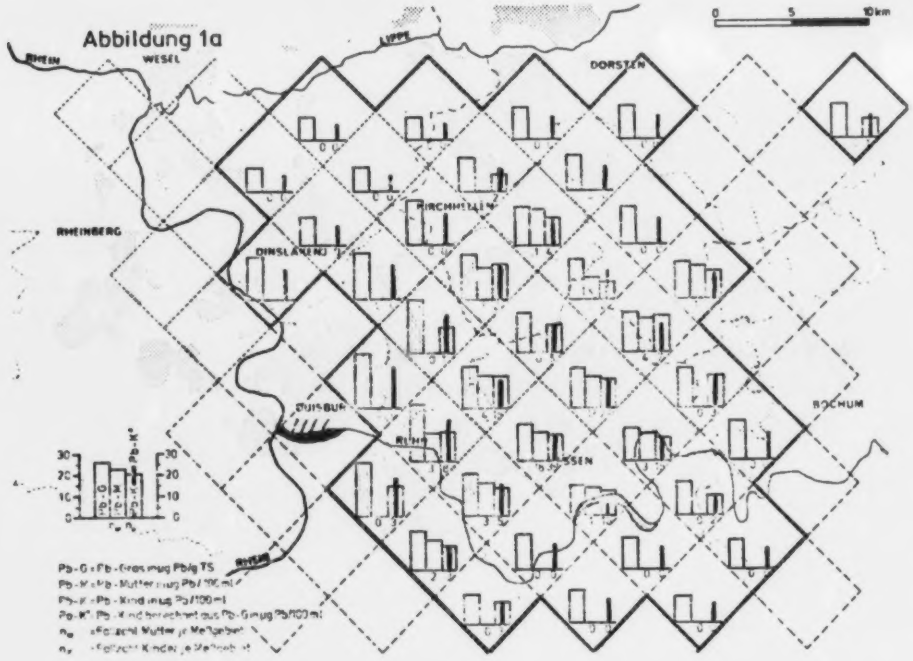
Wiederholt wurde in der Vergangenheit über Organstörungen bei Kindern mit akuter und chronischer Bleiintoxikation berichtet [1, 2]. Diese Beobachtungen lassen vermuten, daß Säuglinge und Kinder besonders empfindlich auf erhöhte Umweltbelastungen mit diesem Schwermetall reagieren. Die Ergebnisse von Menyaz et al. und anderen [3, 4, 5] weisen auf die mögliche Gefährdung des Kindes schon in utero hin. In einem Untersuchungsprogramm zur Erfassung neurologischer Störungen bei Säuglingen mit erhöhtem Blutbleispiegel wurde die Abhängigkeit des Blutbleispiegels von der Bleiimmissionsbelastung der Mutter am Wohnort geprüft.

Methodik

Bei 176 reifen und unreifen Neugeborenen und 59 Müttern mit Wohnsitz im westlichen Ruhrgebiet wurden zwischen Juni 1973 und März 1974 gleichzeitig Untersuchungen des Blutbleispiegels durchgeführt. Ein Teil der Blutproben (Gruppe I) entstammt der Frauenklinik und der Kinderklinik des Klinikum Essen mit einem weit gestreuten Einzugsgebiet. Diese Gruppe enthält auch die unreifen Neugeborenen. Der andere Teil der Blutproben (Gruppe II) entstammt einer gynäkologischen Abteilung (Evang. Krankenhaus Bethesda) im Norden von Essen. In dieser Gruppe weisen die Neugeborenen ein normales Gestationsalter auf. Bei einigen Müttern der Gruppe I erfolgte die Blutprobeentnahme aus technischen Gründen bis zu 2 Wochen post partum. Zur Analyse des Blutes wurde die Methode der flammenlosen Atomabsorptionsspektrometrie verwendet [6, 7]. Die relative Standardabweichung des Verfahrens, ermittelt aus Doppelbestimmungen, beträgt 2,5%.

Die Ergebnisse der Blutbleiuntersuchungen wurden dem Wirkungskataster der Landesanstalt für Immissions- und Bodennutzungsschutz (LIB) zugeordnet. Im Wirkungskataster wird die Bleiaufnahmerate in standardisierten Graskulturen, die an Meßpunkten über eine Gesamtfläche von ca. 1 000 km^2 verteilt sind, ermittelt. Die erhaltenen Werte zeigen die für Menschen, Tiere und Pflanzen gleichermaßen wirksame Bleiimissionsbelastung an [8, 9]. Die Meßpunkte entsprechen den Ecken eines Planquadrates von 25 km^2. Die durchschnittliche Immissionsbelastung für das Planquadrat, in dem sich der Wohnsitz befand, wurde aus dem Mittel der Ergebnisse der vier Eckpunkte errechnet. Bei einigen Quadraten am Rande des Meßgebietes mußte die Immissionsbelastung geschätzt werden. Diese Schätzung erfolgte ohne Vorkenntnis der Ergebnisse der Blutanalyse. Die Bestimmung der Bleiaufnahme im Gras wurde inversvoltammetrisch durchgeführt [10]. Die Ergebnisse sind in Abb. 1a dargestellt.

Abbildung 1a


0 5 10km
RHEIN
WESEL
LIPPE
DORSTEN
RHEINBERG
KIRCHHELLEN
DINSLAKEN
DUISBURG
RUHR
ESSEN
BOCHUM
RHEIN
30 20 10 0
Pb-G
Pb-M
Pb-K
Pb-K*
30 20 10 0
n_M n_K
Pb-G = Pb-Gras mug Pb/g TS
Pb-M = Pb-Mutter mug Pb/100 ml
Pb-K = Pb-Kind mug Pb/100 ml
Pb-K* = Pb-Kind berechnet aus Pb-G mug Pb/100 ml
n_M = Fallzahl Mutter je Meßgebiet
n_K = Fallzahl Kinder je Meßgebiet

Ergebnisse und Diskussion

Bei der Untersuchung der 176 Neugeborenen der Gruppen I und II erhielten wir einen mittleren Blutbleispiegel von 13,3 µg Pb/100 ml und eine Standardabweichung von 4,6 µg Pb/100 ml. Haas et al. [11] haben für ein "Landkollektiv" in der Umgebung Nürnbergs einen gleichen Wert berichtet. Die Verteilung der Fallzahlen in unserer Untersuchung je Meßgebiet sowie die zugehörigen Mittelwerte des kindlichen Blutbleispiegels sind in Abb. 1a dargestellt. In Analogie zu den Untersuchungen von Azar et al. [12] über die Beziehung zwischen der Bleiimmissionsbelastung und dem Blutbleispiegel wurden für den Zusammenhang zwischen der Bleiaufnahmerate im Gras (Pb-G), dem Blutbleispiegel der Mütter (Pb-M) und dem Blutbleispiegel der Neugeborenen (Pb-K) folgende Modelle verwendet:

$$(I) \quad \log Pb\text{-}M = a_{MG} + b_{MG} \log Pb\text{-}G$$

$$(II) \quad \log Pb\text{-}K = a_{KM} + b_{KM} \log Pb\text{-}M$$

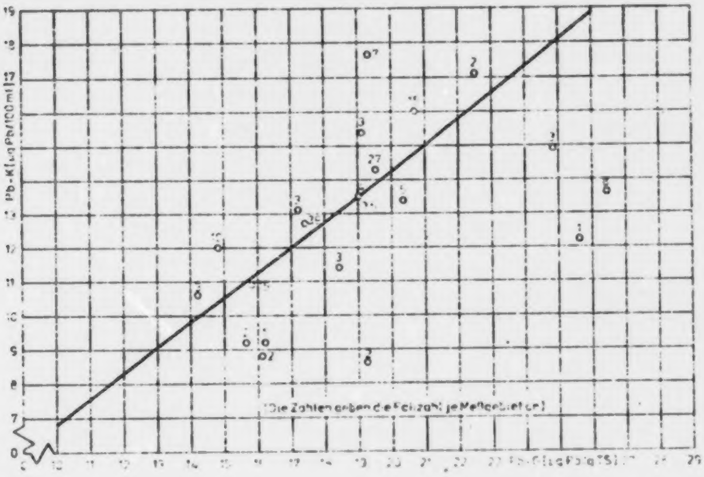
$$(III) \quad \log Pb\text{-}K = a_{KG} + b_{KG} \log Pb\text{-}G.$$

Für den Zusammenhang zwischen dem Blutbleispiegel Kind und der Bleiaufnahmerate Gras entsprechend Modell III wurde für Gruppe I des Gesamtkollektivs ein Korrelationskoeffizient von $r^2 = 0,077$ berechnet. Dieser Koeffizient ist bei n = 118 Kindern auf dem Signifikanzniveau von 99 % gesichert.

Unter den experimentellen Bedingungen des "personal monitoring" fanden Azar et al. [12] einen Korrelationskoeffizienten von $r^2 = 0,436$. Der eigene niedrigere Wert ist durch das grobe Raster des Meßplanes sowie die indirekte Bestimmung der Immissionsbelastung durch ein biologisches System zu erklären.

Nach Elimination der vom Wirkungskataster nicht erfaßbaren räumlichen Heterogenität innerhalb der Meßgebiete, indem statt der Einzelwerte der kindlichen Blutbleispiegel der Mittelwert aller Kinder je Meßgebiet mit dem zugehörigen Immissionswert verglichen wurde, verbesserte sich die Korrelation auf $r^2 = 0,355$ und entspricht damit 81 % des von Azar et al. [12] gefundenen Wertes. Der Zusammenhang ist in Abb. 1b dargestellt.

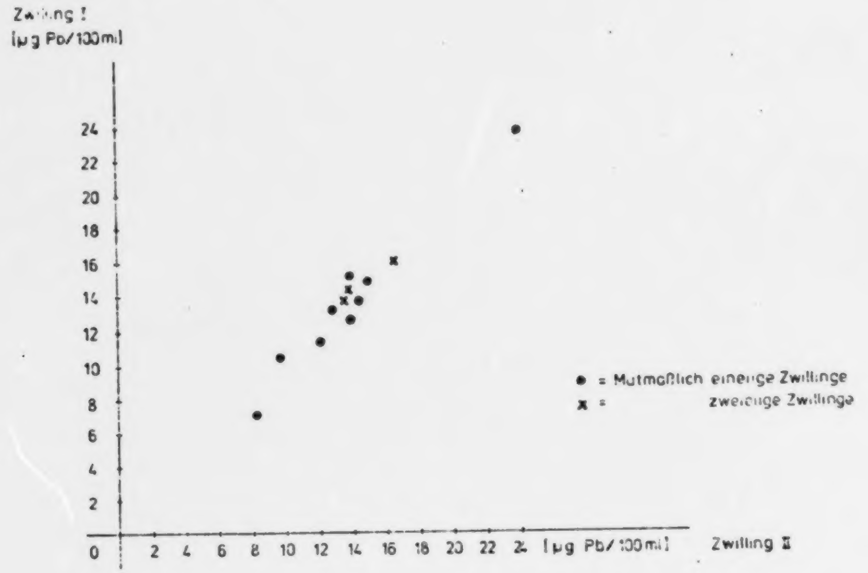
Abb. 1b: Zusammenhang zwischen Blutblei-Kind je Meßgebiet und Blei-Gras



Der mittlere mütterliche Blutbleispiegel beträgt in Gruppe I und II 14,8 µg Pb/100 ml. Die Untersuchungen zum Zusammenhang zwischen kindlichem und mütterlichem Blutbleispiegel entsprechend Modell II ergaben eine Korrelation von $r^2 = 0,323$. Dieser Wert ist bei n = 49 Wertepaaren auf dem Signifikanzniveau von 99 % gesichert. Haas et al. [11] fanden für diesen Zusammenhang einen Korrelationskoeffizienten von $r^2 = 0,290$. In dem von uns beobachteten Verhältnis zwischen kindlichem und mütterlichem Blutbleispiegel von 0,92 besteht eine ähnliche Beziehung wie bei Haas et al. [11], der für sein Stadtkollektiv 0,90 und für sein Landkollektiv 0,87 angibt.

Im Vergleich zu der Mutter-Kind Korrelation sind die Blutbleispiegel bei ein- und zweieiigen Zwillingen mit $r^2 = 0,960$ sehr hoch miteinander korreliert (Abb. 2). Hierbei fügen sich auch die zweieiigen Zwillinge, bei denen ein getrennter Plazentar-

Abbildung 2: Beziehung zwischen dem Blutbleispiegel bei Zwillingen

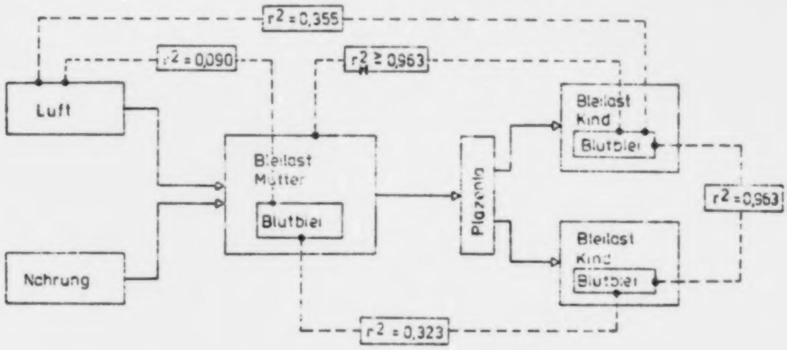


kreislauf angenommen werden muß, in die Korrelation ein. Dies bedeutet, daß zwischen der gesamten mütterlichen Bleilast als verursachender Größe und dem kindlichen Blutbleispiegel bei Kenntnis aller Einflußfaktoren eine Korrelation von wenigstens 0,960 zu erwarten ist. Die Differenz zwischen dieser Korrelation und der Korrelation zwischen mütterlichem und kindlichem Blutbleispiegel bestimmt dabei den Informationsmangel, der zur Klärung der Beziehung zwischen der Bleilast Mutter und dem Blutbleispiegel Kind zur Zeit noch besteht (Abb. 3).

Zur Erfassung weiterer möglicher Einflußfaktoren, die auf den Blutbleispiegel Kind einwirken, wurde die kindliche Bleiaufnahme in Abhängigkeit der kindlichen Blutgruppe geprüft. Wegen des erforderlichen Stichprobenumfanges konnten dabei nur die Blutgruppen A und 0 berücksichtigt werden. Für Blutgruppe A

Abbildung 3: Ursachen der kindlichen Bleibelastung

r^2_H: Hypothetischer Korrelationskoeffizient, abgeleitet aus Zwillingsuntersuchungen



fanden wir bei 55 Kindern einen mittleren Blutbleispiegel von 14,2 µg Pb/100 ml, für Blutgruppe 0 bei 49 Kindern einen Mittelwert von 13,9 µg Pb/100 ml. Die Differenz zwischen beiden Werten ist nicht zu sichern. Die beiden Häufigkeitsverteilungen fallen jedoch außerdem durch eine unterschiedliche Schiefe auf. Für die Blutgruppe A erhält man als Schiefheitsmaß [13] $g_1 = 1,84$, während bei Blutgruppe 0 der Wert $g_1 = 2,28$ beträgt. Sowohl der Mittelwert als auch der Maximalwert der Häufigkeitsverteilung ist somit bei A gegenüber 0 in Richtung höherer Bleiwerte verschoben. Ob die ABO-Antigene eine unterschiedliche Membranpermeabilität für Blei bedingen, muß in weiteren Untersuchungen geklärt werden.

Der Gedanke einer Bleifreisetzung unter Streß, Schwangerschaft und Laktation wird in der Literatur diskutiert [14], ohne daß unseres Wissens über erste Ergebnisse berichtet wurde. Bei der Analyse der Untersuchungsgruppe I wurde eine Beziehung zwischen dem log-Gestationsalter und dem Blutbleispiegel von $r^2 = 0,076$ bei $n = 30$ ermittelt. Dieser Zusammenhang ist bei Annahme einer Irrtumswahrscheinlichkeit von 10 % knapp gesichert. Der Befund stützt die in der Literatur diskutierte Hypothese über die Bleifreisetzung während der Schwangerschaft. Bei Erweiterung des Kollektivs auf die Gruppe II wurde die Korrelation schwächer. Dies ist vermutlich auf das normale Gestationsalter der Kinder der Gruppe II zurückzuführen. Eine Beziehung zwischen dem log-Gestationsalter und dem Blutbleispiegel der Kinder war nicht zu sichern.

Ein Zusammenhang zwischen der Bleiaufnahmerate Gras und dem mütterlichen Blutbleispiegel bei Gruppe I und II entsprechend Modell I konnte zunächst nicht nachgewiesen werden. Erst nach Elimination der räumlichen Heterogenität durch Mittelwertbildung je Meßgebiet und Wichtung dieser Werte mit den entsprechenden Besetzungszahlen erhält man eine Korrelation von $r^2 = 0{,}090$ (Abb. 3). Der im Vergleich zum Kind-/Gras-Verhältnis niedrige Korrelationskoeffizient läßt sich auf die geringe Besetzungszahl je Meßgebiet zurückführen, durch die die räumliche Repräsentanz erheblich eingeschränkt wird. Die räumliche Zuordnung der Ergebnisse ist in Abb. la dargestellt.

Wir danken Herrn Prof. Ludwig, Direktor der Frauenklinik der Gesamthochschule Essen, und Herrn Dr. Pomp, Direktor der gynäkologischen Abteilung am Evangelischen Krankenhaus Bethesda in Essen, sowie Herrn Dr. Scholl von der LIB für die Unterstützung des Untersuchungsprogramms.

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DISKUSSION

KREUZER (Bundesrepublik Deutschland)

Mich überrascht die von Ihnen ermittelte Korrelation zwischen dem Blut-Pb des Kindes und dem Bleigehalt im Gras etwas, wenn man die vielen die Pb-Kontamination der Pflanzen beeinflussenden Faktoren in Betracht zieht, wie z.B. den Einfluss der Pflanzenspezies, ihrer Oberflächenbeschaffenheit sowie den der Niederschläge (langfristiges Niederschlagsmittel, Niederschlagshäufigkeit) auf oberflächlich abgelagertes Blei. Niederschläge können (in Abhängigkeit von der Art - Schauer, Nieseln -) erhebliche Mengen oberflächig auf Pflanzen abgelagertes Blei entfernen.

Fragen: I) Welche Gräser (Spezies) wurden auf ihren Pb-Gehalt untersucht?
2) Welche Unterschiede in der Niederschlagsverteilung bestanden im Untersuchungsgebiet?

HOWER (Bundesrepublik Deutschland)

Zur Bestimmung der Bleiaufnahmerate im Gras der Spezies Lolium multiflorum wurde ein standardisiertes Verfahren benutzt, (Scholl, G.: Staub- Reinhaltung der Luft, 34 : 89-92, 1974), bei dem die Expositionsbedingungen - Anzucht, Bodensubstrat, Bodenfeuchte etc.. - weitgehend konstant gehalten werden. Zur Analyse der Bleiaufnahmerate wurden gewaschene Proben verwendet, so dass lediglich das inkorporierte, von Niederschlägen weniger abhängige Blei bestimmt wurde. Die meteorologischen Bedingungen innerhalb des Ueberwachungsgebietes von etwa 1000 km2 Grösse sind zudem fast gleich.

INFLUENCE DE L'EXPOSITION AUX GAZ D'ECHAPPEMENT AUTOMOBILES SUR L'IMPREGNATION PAR LE PLOMB DE DIFFERENTS GROUPES DE POPULATION

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RESUME

L'utilisation du plomb têtra-éthyle et du plomb tétraméthyle comme antidétonants dans les carburants est à l'origine de la présence du plomb minéral dans les gaz d'échappement automobiles. La fine granulomètrie des particules plombifères (75% auraient un diamètre moyen infèrieur au micromètre), leur forte teneur en plomb, ainsi que le rendement important (30 à 50% selon les auteurs) de la pénètration par voie pulmonaire, font que l'apport dû à la respiration de 15 à 20 m^3 *d'air par 24 heures, s'ajoutant au plomb amené par l'alimentation, est loin d'être négligeable.*

Nous avons voulu mettre en évidence l'influence éventuelle de ce plomb d'origine atmosphérique grâce à la détermination de la plombémie et de l'activité ALA-D érythrocytaire chez 3 groupes de population exposés à des concentrations de plomb dans l'air nettement différentes.

Après avoir envisagé les caractéristiques de ces divers groupes, nous indiquerons succinctement les techniques utilisées, avant d'aborder l'exposé des résultats obtenus.

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ABSTRACT

The use of tetraethyl and tetramethyl lead as anti-knock additives in petrol is responsible for the presence of mineral lead in motor car exhaust fumes. Owing to the fineness of the lead-bearing particles (75% are reported to have an average diameter of less than one micron), their high lead concentration and the high rate of absorption (30% to 50% according to the authors) through the lungs, the lead absorption resulting from the respiration of 15 to 20 m^3 *per 24 hours, together with lead intake with food, is far from negligible.*

We have sought to reveal the possible influence of this lead of atmospheric origin by determining the lead content of the blood and the ALA-D erythrocyte activity in three population groups exposed to markedly different lead concentrations in the air.

After considering the characteristics of these different groups, we shall describe briefly the techniques used and shall then proceed to outline the results obtained.

A. CARACTERISTIQUES DES GROUPES DE POPULATION CHOISIS.

- GROUPE I : GROUPE RURAL.

constitué de 95 sujets (dont 55 % de femmes) vivant dans 2 villages du département des Yvelines, Longnes et Dammartin, situés à environ 80 km à l'ouest de Paris. Les prélèvements ont été réalisés à l'occasion de collectes organisées par le centre de Transfusion Sanguine des Yvelines-Nord (Dr. CULOT)

- GROUPE II : GROUPE URBAIN NON SPECIALEMENT EXPOSE.

constitué de 5I sujets (dont 29 % de femmes) habitant et travaillant à Paris ou sa proche banlieue, pris parmi les volontaires se présentant à la Banque du Sang du groupe hospitalier Pitié-Salpêtrière (Pr. TZANCK). Précisons que, comme d'ailleurs dans le cas du groupe I, il n'a pas été réalisé de choix avec critère professionnel, puisque nous avons voulu étudier des groupes réflètant l'ensemble d'une population,avec l'hétérogénéité que cela suppose. Nous avons cependant écarté les sujets pouvant être considérés comme spécialement exposés (chauffeurs routiers, employés de garage...)

- GROUPE III : CHAUFFEURS DE TAXI.

constitué par 58 chauffeurs d'une société de taxis parisiens, dont 56 du sexe masculin. Tous résidaient à Paris ou sa proche banlieue et assuraient chaque jour environ I0 heures de conduite. Les prélèvements ont été réalisés au service médical de la société (Docteur LAFONT) avant le début de la journée de travail. L'ancienneté professionnelle de ce groupe était de 7.8 années. 60 % des sujets étaient fumeurs, mais seulement 28 % d'entre eux consommaient plus de I5 g de tabac par jour.

B. TECHNIQUES

I - Détermination de la teneur en plomb de l'air atmosphèrique.

Dans le cas de la zone rurale, nous avons utilisé la méthode que nous avons antérieurement décrite (I). Le prélèvement est effectué sur un filtre en esters de cellulose, type Millipore, à l'aide d'un "low volume sampler" (débit environ 2 m3/24 H.). Le filtre est ensuite soumis à l'action dissolvante et oxydante de l'acide nitrique en présence d'une faible quantité d'acide perchlorique. Après évaporation totale, on reprend par un volume exactement connu de solution d'HNO_3 à I%. La teneur en plomb de cette liqueur de reprise est ensuite déterminée par AAS sans flamme.
Pour la détermination de la teneur moyenne dans l'air de la cabine d'un taxi,nous avons utilisé un appareil autonome, fonctionnant sur batterie d'accumulateurs et muni d'un filtre Millipore identique à celui de la technique précèdente. Les prélèvements ont porté sur une centaine d'heures (environ 4 m3) et les filtres ont été traités comme ci-dessus. Pour Paris, nous avons utilisé les résultats publiés pour I972 par le laboratoire de la Préfecture de Police.

II - Détermination de la plombémie.

Effectuée par AAS sans flamme (four de graphite Perkin-Elmer), après digestion de la prise d'essai de sang (0.5 ml) par l'acide nitrique à l'étuve à 75°C, dans des tubes de polystyrène à usage unique, obturés à l'aide d'un bouchon de polyéthylène (Technique en cours de publication).

III - Détermination de l'activité ALA D érythrocytaire.

Cette détermination a été effectuée selon la technique manuelle de VALLEE (2). Le sang est recueilli sur héparinate de lithium, puis hémolysé et incubé pendant 30 minutes à 38°C en présence de tampon TRIS (pH = 7.0) avec une solution d'ALA 0.00782 M. Les résultats sont exprimés en "unités Vallee". Selon l'auteur, les valeurs normales seraient de I5.9 ± 4.3 U.

C. RESULTATS.

I - Teneur en plomb de l'air.

a) Zone rurale (Gr. I)

Le contrôle a été effectué pendant une année complète, de mai I972 à mai I973, avec pour chaque prélèvement une durée de 7 jours. Les moyennes annuelles trouvées ont été de:

Dammartin : 0.35 ± 0.22 µg/ m3
Longnes : 0.26 ± 0.I7

Il s'agit donc bien, selon le critère généralement admis (teneurs moyennes inférieures à 0.5 µg / m3) d'une zone de caractère rural. Les teneurs mensuelles ont toujours été trouvées inférieures à 0.5, sauf pour juillet et septembre I972, à Dammartin.

b) Paris (Gr. II)

Moyennes annuelles de I972

Rond-Point des Champs-Elysées :	2.70 µg/m3
Place V. Basch	2.80
Moyenne calculée sur l'ensemble des 5 postes dépendant de la Préfecture de Police	I.98

c) Détermination de la teneur moyenne en plomb (portant sur environ I00 h.) de l'air de la cabine d'un taxi.

Les mesures ont été réalisées en juillet I973, ainsi qu'en octobre, mois pendant lequel la circulation parisienne est traditionnellement intense. Dans les 2 cas, les teneurs obtenues sont très peu différentes de 3 µg/ m3

II - Paramètres biologiques.

Gr. I	moy. arith.	écart-type
Pb/sang (x) N= 93	27.8 µg/I00 ml	9.98
ALA D (y) N = 95	II.7 U. Vallee	3.76

r = - 0.70
y = - 0.26 x + I9.I8

Gr. II N = 5I	moy. arith.	écart-type
Pb/sang (x)	29.4	I0.88
ALA D (y)	II.48	3.97

r = - 0.62
y = - 0.23 x + I8.I5

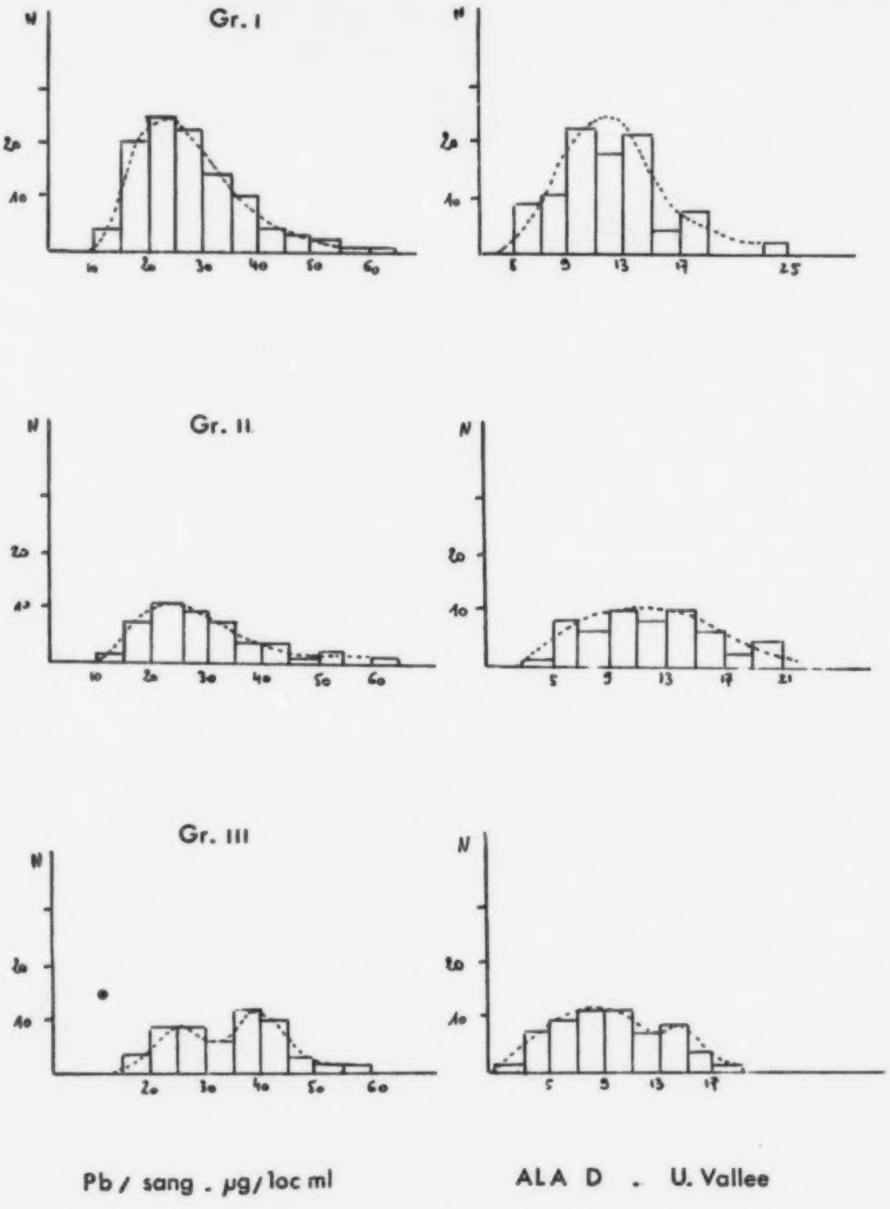

Figure 1 : Variation de la plombémie et de l'activité ALAD dans les trois groupes de population

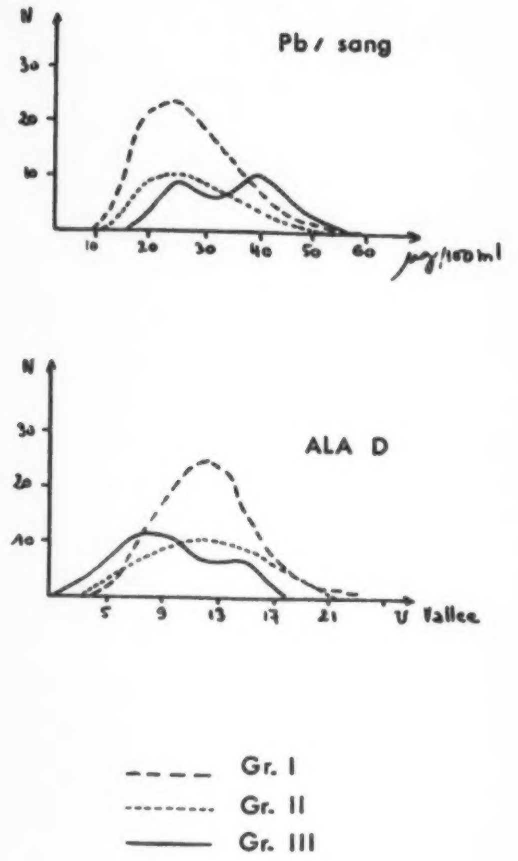

Figure 2 : Comparaison pour les trois groupes de population des variations dans le taux de plombémie et de l'activité ALAD

Gr. III N = 58	moy.arith.	écart-type
Pb/sang (x)	34.7	IO.I5
ALA D (y)	9.4	3.73

$$r = - 0.64$$
$$y = - 0.24\,x + I7.68$$

D. DISCUSSION.

L'examen des tableaux de résultats, ainsi que des schémas correspondants, amène les observations suivantes: (Fig 1 et 2)
I) par application du test t de Student à la comparaison des moyennes obtenues pour les différents groupes (dans la mesure où ce test peut être appliqué à des distributions aussi peu classiques), il n'apparait pas de différence statistiquement significative entreles groupes I et II, tant du point de vue de la plombémie que de l'activité ALA-D, bien que les niveaux correspondants d'exposition au plomb atmosphèrique soient très différents (souvent dans un rapport de I à IO). Par contre, il y a une différence très significative ($P < 0.0I$) entre le groupe III d'une part, et chacun des groupes I et II d'autre part.

Cette observation peut paraitre a priori étonnante, dans la mesure où les taux de plomb dans l'air indiqués pour les groupes II et III paraissent identiques. Il faut souligner toutefois que le taux de I.98 µg/m3 donné comme moyenne annuelle pour Paris en I972 est la moyenne arithmétique des taux observés au niveau de 5 points de prélèvement situés majoritairement dans des endroits particulièrement pollués où ne séjournent pas en permanence les parisiens, alors que le taux de 3 µg/ m3 pris comme taux de référence pour ce groupe III représente de manière beaucoup plus réaliste la concentration respirée par un chauffeur de taxi pendant une fraction importante de la journée (IO heures).

Ces résultats qui paraissent traduire une imprégnation certes discrète, mais cependant plus importante des chauffeurs de taxi par rapport à l'ensemble de la population, sont en accord avec les résultats obtenus aux USA par AZAR et coll. (3), mais diffèrent par contre de ceux obtenus en Grande-Bretagne par JONES, COMMINS et CERNIK (4). Leur interprètation mérite cependant d'être plus nuancée en tenant compte de la répartition inégale des sexes dans les trois groupes. En effet, nous signalons plus loin les différences significatives trouvées pour la plombémie entre les hommes et les femmes à l'intérieur de chaque groupe. Si l'on compare les plombémies des seuls hommes entre les différents groupes, il n'y a plus de différence significative entre les groupes I et II, et il n'existe plus qu'une différence faiblement significative ($P > 0,5$) entre les groupes III et I d'une part et III et II d'autre part.
II) les moyennes arithmètiques des plombémies et notamment celles du groupe I sont nettement supérieures à celles trouvées dans un certain nombre d'autres pays. Rappelons à ce sujet qu'en I967 GOLDWATER et HOOVER avaient trouvé le taux de I7 µg/IOO ml de sang, comme moyenne calculée sur des échantillons prélevés dans I4 pays différents (5). Cette particularité que la France semble partager avec l'Italie (6) s'explique à notre avis par la consommation importante de vin dans ces 2 pays. L'un de nous a pu montrer en effet avec TRUHAUT et ALBAHARY (7) que le vin constituait un apport non

négligeable de plomb à notre alimentation. On peut supposer que ce sont des différences individuelles dans les habitudes alimentaires, de ce fait plus marquées en France que dans d'autres pays, qui seraient responsables d'une plus grande dispersion des résultats. D'ailleurs l'examen des histogrammes des plombémies du groupe I permet de constater qu'en fait le maximum de fréquence de leur répartition se situe entre 20 et 25 µg, c'est à dire dans une fourchette très voisine de celle observée dans les autres pays. Il en est de même pour le groupe II mais par contre le groupe III parait montrer une répartition bimodale difficilement expliquable, sinon par l'existence au sein de ce groupe critique d'un sous-groupe plus particulièrement exposé, pour des raisons vraisemblablement professionnelles.
III) dans le cas des groupes I et II au sein desquels il nous a été possible de dissocier les résultats suivant le sexe des sujets, nous avons observé une moyenne de plombémie plus faible chez les femmes.

	Pb / sang		ALA D	
	hommes	femmes	hommes	femmes
Gr.I	3I.5 ± 8.8	26.2± I0.5	I0.3 ± 3.2I	I2.53 ± 3.6I
Gr.II	32.8 ±I0.6	2I.3± 6.39	I0.5 ± 3.48	I3.76 ± 4.27

Cette particularité qui a été décrite également par SECCHI et coll. (6) pourrait s'expliquer par une consommation moindre de vin. Il faut signaler toutefois que cette différence qui n'était pas apparente dans l'enquête de GOLDWATER et HOOVER (5) a été cependant retrouvée aux USA par HOFREUTER et coll. (8).
IV) nous avons retrouvé dans chaque groupe une corrélation négative entre la plombémie et l'activité ALA D : les coefficients de corrélation sont voisins et les équations des droites de régression pratiquement identiques. Enfin, après d'autres auteurs (3, 6) nous avons retrouvé une moyenne d'activité ALA D plus élevée chez les femmes; mais ceci n'est peut-être que la simple conséquence d'une plombémie plus faible.

CONCLUSION.

D'après les déterminations de plombémie et d'activité ALA D qui sont actuellement reconnues comme les critères d'imprégnation saturnine les plus valables et les plus sensibles, il n'y a pas de différence significative entre un groupe de population parisienne et un groupe de population rurale prise comme témoin. Par contre ces 2 groupes montrent, lorsqu'ils sont pris dans leur ensemble, une différence significative par rapport à un troisième groupe de chauffeurs de taxi, différence apparaissant d'ailleurs nettement moins significative si l'on ne fait porter la comparaison que sur les hommes.

Et cependant la concentration de plomb dans l'air respiré par ce 3° groupe est du même ordre de grandeur que celle qui est habituellement indiquée comme moyenne annuelle pour la ville de Paris, alors que la concentration déterminée en zone rurale est environ I0 fois plus faible!...

Ceci tend à prouver que le groupe urbain n'est pas, dans l'état actuel des choses, plus imprégné par le plomb que le groupe rural, malgré une concentration atmosphérique apparemment plus élevée. Ces observations posent le problème de la représentativité,du point de vue de la santé publique, de prélèvements effectués dans les rues à grande circulation et montre la nécessité de multiplier les dosages à l'intérieur des habitations.

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DISCUSSION

BERLIN (C.E.C.)

Dans votre communication vous montrez qu'il semble y avoir (d'après les niveaux de plombémie et d'ALAD) une exposition plus grande au plomb présent dans l'environnement pour les chauffeurs de taxi que pour des populations rurales et urbaines non spécialement exposées.

A quel facteur attribuez vous les plombémies plus basses et les niveaux d'ALAD supérieurs observés chez les femmes habitant des zones urbaines en comparaison avec celles habitant à la compagne? Les différences entre les plombémies 26,2 μg/100 ml contre 21,3 μg/100 ml sont-elles significatives?

BOUDENE (France)

La différence qui existe entre les moyennes trouvées, pour la plombémie,

d'une part, pour les sujets féminins du groupe I rural (26,2 ± 10,5)
et d'autre part, pour les sujets féminins du groupe II urbain (21,3 ± 6,39)
n'est que très faiblement significative
(significatif au seuil de P = 0,5).

La seule explication que nous pourrions peut être avancer serait que les femmes du groupe I, étaient dans l'ensemble nettement plus âgées que les femmes du groupe II, qui étaient pour la plupart des étudiantes ou de jeunes infirmières du groupe hospitalier Pitié-Salpêtrière.

HOLL (Republique fédérale d'Allemagne)

Vous avez fait un exposé impressionnant de la façon dont les chauffeurs de taxis réagissent aux concentrations de Pb de l'air des grandes villes. Dans l'hypothèse où ils inhalent aussi d'autres substances, par exemple SO_2, NO, NO_x, de même que des éléments de fumée de tabac, si les clients fument - peut-on, d'après l'expérience acquise en France, admettre que les chauffeurs de taxis sont plus exposés, en raison de la composition des polluants de l'air, même si la ventilation du vehicule est assurée correctement?

BOUDENE (France)

Il faut tout d'abord préciser de nouveau que nous n'avons trouvé qu'une différence faiblement significative entre le Groupe II (urbain, hommes seulement) et le Groupe III (chauffeurs de taxi).

Si nous n'avons pas d'expérience propre en ce qui concerne une éventuelle exposition augmentée de ces chauffeurs à SO_2 et NO_x, nous avons par contre effectué la détermination de l'oxycarbonémie pour ce Groupe III.

Cette oxycarbonémie est, pour ce groupe que l'on peut effectivement supposer légèrement plus exposé aux gaz d'échappement, directement fonction de la quantité de tabac fumée, ce qui est tout à fait habituel.

Si l'on considère les chauffeurs non fumeurs stricts (40% de l'ensemble), l'oxycarbonémie est cependant légèrement supérieure (0,36 ± 0,13 ml de CO/100 ml sang) à ce que nous avons trouvé pour un groupe témoin (non fumeurs stricts, non chauffeurs de taxi) (0,24 ± 0,11 ml CO/100 ml sang).

INVESTIGATIONS OF THE ABSORPTION OF SOME METALS AMONG PEOPLE IN THE SURROUNDING AREA OF A SMELTING PLANT

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ABSTRACT

The investigation was conducted in order to establish the extent to which the emissions of heavy metals from a smelting plant could give rise to excessively high levels of these metals in the blood and urine of people living in the surrounding area and whether or not any biological effects of the emissions could be observed.

Analyses were made of the levels of the metals in the urine of women from 3 groups living at different distances and in various directions from the smelting plant. Arsenic, lead, cadmium and mercury levels were measured. The analysis did not detect any abnormally high levels of these metals in the urine of the women from any of the groups. Some difference in arsenic levels between the groups could be detected. This difference was due to meteorological factors. The levels of lead, cadmium and mercury found in the samples were low, and there were no differences between the various regions. Tubular protein was not found, either in cadmium-exposed workers from the smelting plant or in women from the surrounding area. The metals emitted from the smelting plant do not appear to give rise to hazardous levels of these metals in the urine of the people living in the surrounding area.

Lead determinations conducted on blood gave a somewhat higher mean value for men who lived in the immediate vicinity of the smelting plant (mean value = 13.6 µg/100ml) than for men who lived further away from the plant. Students exhibited a mean level (11.3 µg/100ml) which was significantly lower than that exhibited by working men (mean = 13.5 µg/100ml). Among the working men, metal workers had the highest mean value (15.9 µg/100ml) while workers at wood and pulp mills had the lowest (11.9 µg/100ml). Students at trade schools exhibited significantly higher values than other categories of students. Trade school students: Mean = 11.8 µg/100ml. Other students: Mean = 10.40 µg/100ml.

Profession seems to be more important for blood lead level than distance from the smelting plants. Age seems to be of no importance.

No differences in the ALA content of the urine of the groups mentioned above were found, even though the difference in the level of lead in the blood amounted to 8.8 µg/100ml. The ALA values hovered at about 0.30mg% for all groups, with no significant differences between groups.

1. Introduction

All smelting plants, including the Boliden Company's Rönnskär Works, emit certain substances into the surrounding area. See map 1 and 2. Heavy metals are among these emitted substances. The presence of these substances in the environment has been detected and measured by analyses of the precipitation in the area, measurements of metal levels in animal tissues and examination of the mosses and other plants growing in the area surrounding the smelting plant.

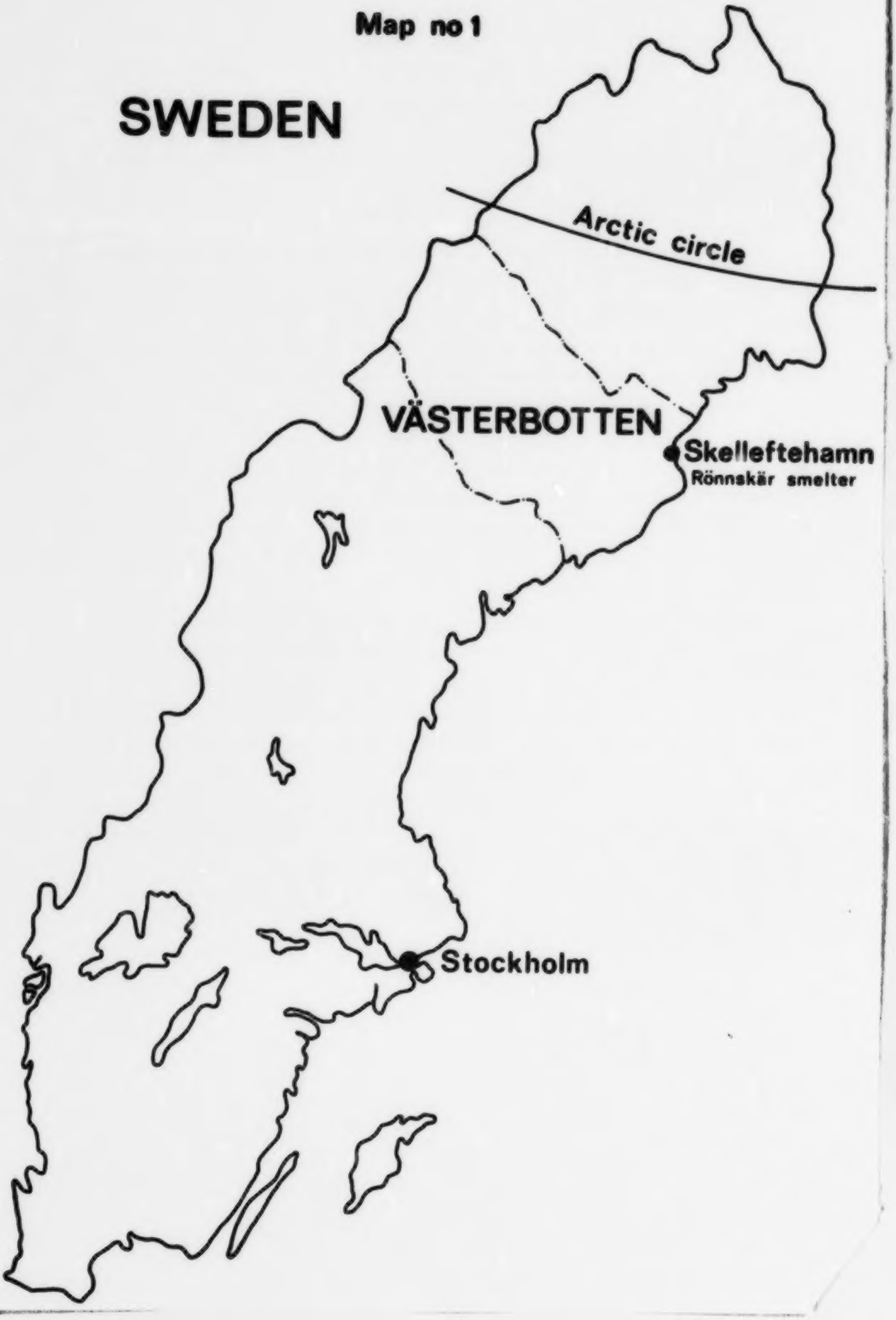
Little previous work has been done on the effect of these emissions on human beings, and in order to evaluate this effect, it is necessary to get an idea of the extent to which the substances in question have been assimilated by the human body. This can be done by means of blood and urine analyses. Once this knowledge is acquired, the effects of the assimilation of these substances can then be studied in various ways, such as by investigating the effect of cadmium assimilation on the occurrence of tubular protein, and the occurrence of ALA in the blood in connection with lead accumulation.

2. Urine analyses for arsenic, cadmium, lead and mercury

This paper is the report of an investigation conducted in 1973, in cooperation with the Skellefteå Public Health Board and the environmental hygiene institute of the Swedish Environment Protection Board. The investigation was concerned with the determination of the levels of arsenic, lead, cadmium and mercury in urine.

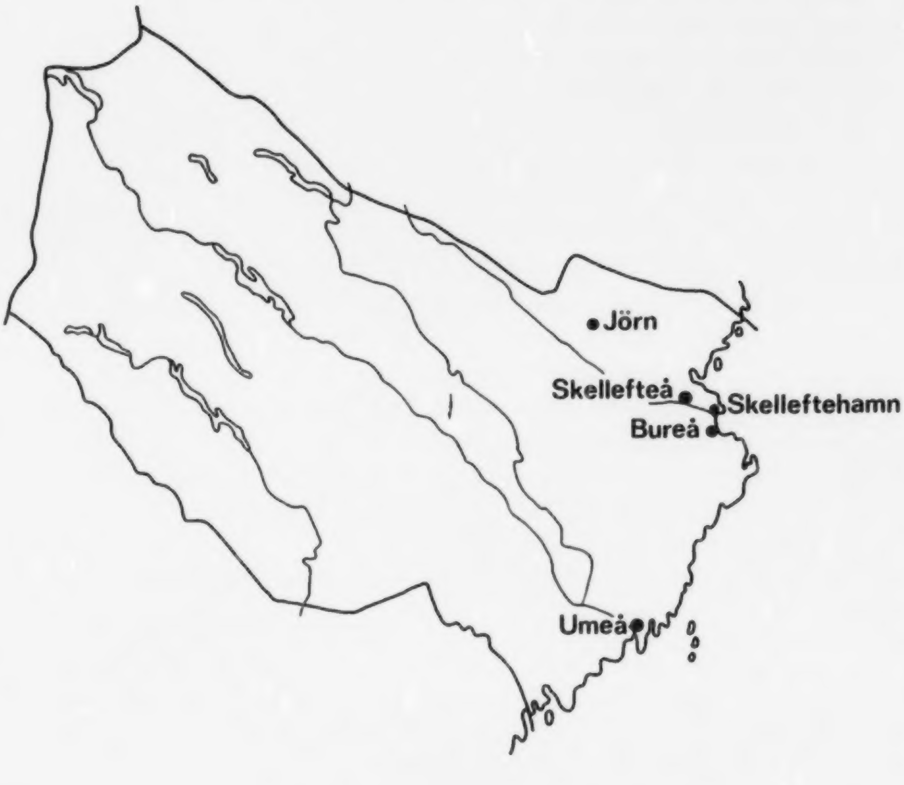
The urine analyses for arsenic, lead, and mercury were performed in our own laboratory. The cadmium analyses were performed by Professor Friberg at the environmental hygiene institute of the Swedish Environment Protection Board. Besides cadmium analyses, studies of the occurrence of tubular protein were made, and some of the circumstances surrounding cadmium exposure were elucidated.

Map no 1


SWEDEN
Arctic circle
VÄSTERBOTTEN
Skelleftehamn
Rönnskär smelter
Stockholm

Map no 2

VÄSTERBOTTEN COUNTY



Urine samples were collected from women from three districts in the surrounding region: *Skelleftehamn*, within 5 km in a westward direction from the Rönnskär Works, *Bureå*, slightly more than 5 km south of the works, and *Jörn*, more than 60 km from the smelting plant, which served as a reference district. Women were chosen for the investigation because they were assumed to comprise a more homogeneous group than men from the standpoint of exposure, since men normally move around more in the course of their work or as a result of changing jobs and are thereby subjected to more variable cadmium exposure. The women studied in the investigation had resided in the same communities for at least 30 years. The reason the sample group was restricted in this way is that it takes such a long time for tubular protein to appear in the urine. Urine samples were collected from 28 women in Skelleftehamn, 30 women from Bureå and 30 from Jörn. In addition, 30 lead workers from the Rönnskär Works were included in the study. These workers had been exposed to lead and to some extent cadmium for 15-30 years. Thus, 4 groups numbering a total of 118 persons were studied.

The samples were collected by district nurses in each community and then turned over to the Public Health Board, where they were coded and then given to the laboratories. As a result of this procedure, the analysis personnel did not know where the samples came from. Thus, the investigation was "blind".

Then, when the laboratories had finished testing, the results were presented to the Public Health Board, which revealed the code and then compiled the results.

The results of the urine analyses are shown in Table 1.

Table 1

Arsenic, cadmium, lead and mercury levels in urine

		Pb in urine µg/1000 ml				Hg in urine µg/1000 ml
Region	N	Range	Median	Mean	N	Range
Rönnskär	30	<5-118	48	≈53	30	<2-4
Skelleftehamn	28	<5-16	8	≈ 8	28	<2-4
Bureå	30	<5-24	7	≈ 9	30	<2 all
Jörn	30	<5-23	7	≈ 7	30	<2-3

		As urine ug/1000 ml				Cd in urine ug/g creatinine		
Region	N	Range	Median	Mean	N	Range	Median	Mean
Rönnskär	30	<20-90	31	≈43	30	0.1-1.4	0.38	0.55
Skelleftehamn	28	<20-70	30	≈29	28	0.1-1.9	0.54	0.61
Bureå	30	<20-110	41	≈49	30	0.2-2.0	0.60	0.71
Jörn	30	<20-95	7	≈33	30	0.1-1.9	0.52	0.66

As was expected, the lead workers had much higher lead excretion than the other three groups. The differences between the lead levels in the urine from the women from Skelleftehamn, Bureå and Jörn are not statistically significant and the levels are very low. It therefore appears improbable that women from these three regions have an elevated rate of lead absorption. (See Cantarow and Trumper, as well as Kehoe, for information on the normal values of lead levels in urine and corresponding values for persons exposed to lead.)

The mercury levels in all groups were low, and there were no statistically significant differences between the groups. This evaluation is based on the comparisons of urine levels done by Friberg and by Tejning and Öhman.

As far as Rönnskär is concerned, previous urine analyses of mercury, performed both at the National Institute for Occupational Health and at our own laboratory, as well as measurements taken of the air in the works, have not shown elevated levels.

There were some differences in arsenic excretion between the four groups. The lead workers in Rönnskär and the women from Bureå had significantly higher values than the women in Skelleftehamn and Jörn. There was no difference between Rönnskär and Bureå or between Skelleftehamn and Jörn. When we try to draw conclusions regarding arsenic exposure based on the urine levels, we must remember that the lead workers at Rönnskär have little exposure to arsenic. The workers in the departments which process arsenic display completely different and higher values. However, the women from Bureå must be considered a highly exposed group if we take into account non-industrial exposure. When the winds are from the north, Bureå can be highly exposed. However, the arsenic levels in the urine samples from all groups were within acceptable limits.

The low values for the women in Skelleftehamn, who live so close to the source of emissions, appear confusing at first, but they can probably be explained by the fact that easterly winds are relatively uncommon, and by the fact that the stack on the Rönnskär Works is so high that when the wind is from the east, Skelleftehamn lies, so to speak, below the line of fire.

As early as 1918, Bang determined the normal value for arsenic in urine. He also showed how the level of arsenic in the urine varies considerably from day to day and pointed out the importance of diet. G. Westöö and M. Rydälv have reported instances of a substantial elevation of the urine arsenic level after the consumption of seafood (plaice) containing arsenic. (See the handbooks by Koelsch and Patty for further information regarding the evaluation of the level of arsenic in urine.)

The results indicated that there was no difference in the level of cadmium in the urine from the four groups included in the study. Nor was any tubular protein found.

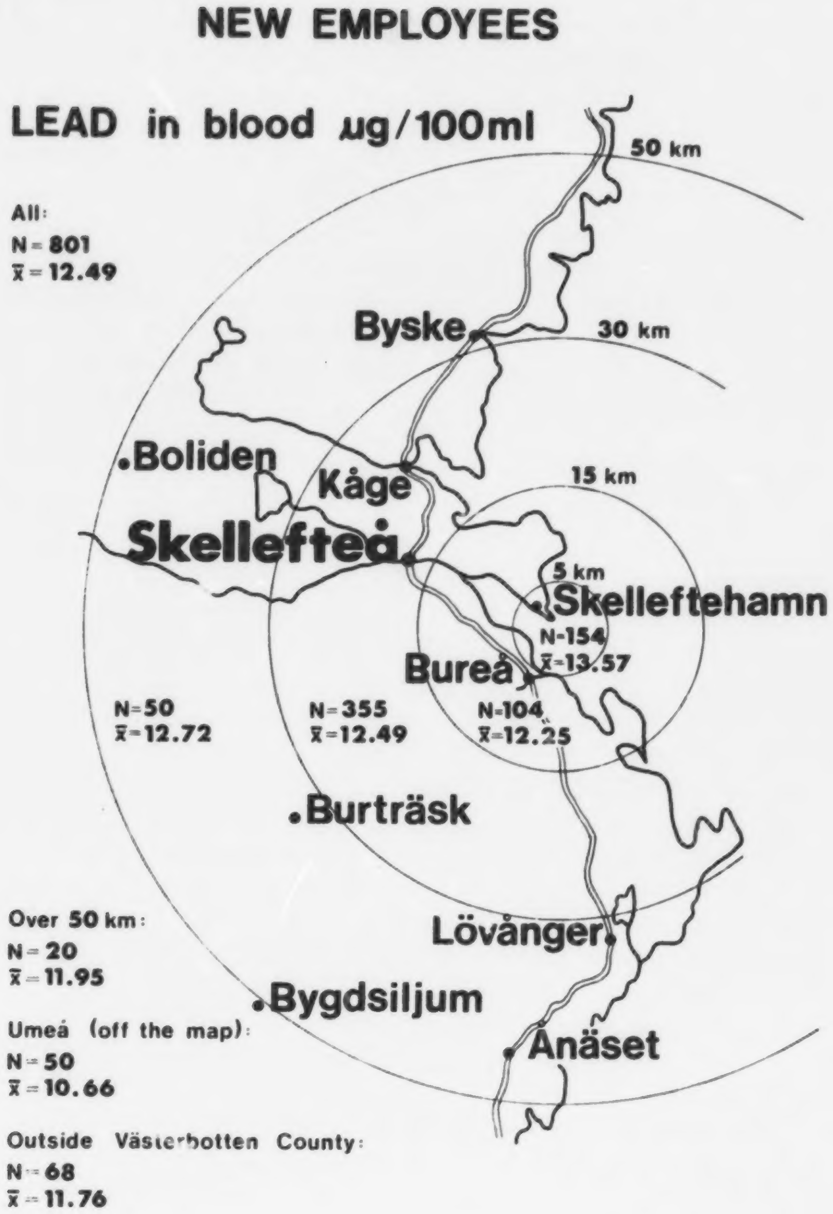
In summary, it may be said that the procedures used in the investigation failed to indicate any health hazards caused by the substances studied in the area surrounding the smelting plant.

3. Lead in blood and ALA in urine

Lead tests on blood samples taken from men in the surrounding area have been performed since 1950 at the Rönnskär Works in connection with the hiring of new employees. The material resulting from these studies was reported at the occupational medicine conference in Vienna in 1966 (Holmqvist). The studies have continued since then, and ALA determinations in the urine were begun in 1968.

The lead level values from the blood of 801 men living at various distances from the smelting plant are shown in Fig. 1. These distances have been divided into regions of < 5, 5-15, 15-30, 30-50 and > 50 km from the source of emissions. In addition, a large number of men from Umeå and from outside Västerbotten County have

Fig 1


NEW EMPLOYEES
LEAD in blood µg/100ml
All:
N=801
x̄=12.49
50 km
30 km
15 km
5 km
Byske
Boliden
Kåge
Skellefteå
Skelleftehamn
N-154
x̄=13.57
Bureå
N=50
x̄=12.72
N=355
x̄=12.49
N-104
x̄=12.25
Burträsk
Over 50 km:
N=20
x̄=11.95
Lövånger
Bygdsiljum
Ånäset
Umeå (off the map):
N=50
x̄=10.66
Outside Västerbotten County:
N=68
x̄=11.76

also been studied.

The highest value, 13-57 µg/100 ml blood, was found in men residing at a distance of less than 5 km from the Rönnskär Works. It is clear from the statistical treatment that this value is significantly higher than all the other values. The mean values within the region 5-50 km are significantly higher than those more than 50 km away - outside Västerbotten County. The differences between the three groups within the region 5-50 km are not significant.

The corresponding ALA values are reported in Fig. 2. There are no significant differences.

In the results of the Vienna report, the students had lower levels of lead in their blood than the working men. The material has now been divided up into students and working men, and it can be seen from table 2 that working men have a higher mean lead level in their blood than students.

Table 2
Lead in blood, (µg/100 ml), from students and working men

	n	Range	$\bar{X}$
All	801	4-62	12.49
Students	369	4-40	11.26
Working men	432	4-62	13.50

However, there is no such difference in the ALA values (Table 3).

Table 3
ALA levels in urine (mg/100 ml) from students and working men

	n	Range	$\bar{X}$
All	790	0.05-2.08	0.3030
Students	361	0.05-2.08	0.3099
Working men	429	0.05-0.87	0.2972

Fig. 3 reports the average lead levels in the blood of students living at various distances from the source of emissions. The mean value for students living within 0-50 km was significantly higher than for those living more than 50 km away - outside Västerbotten County. "Umeå" is not significantly lower than "50 km" - "Outside Västerbotten County".

The students in all the regions had lower levels of lead in their blood than working men.

No difference in mean ALA levels could be detected between students and working men at various distances from the smelting

Fig 2

NEW EMPLOYEES

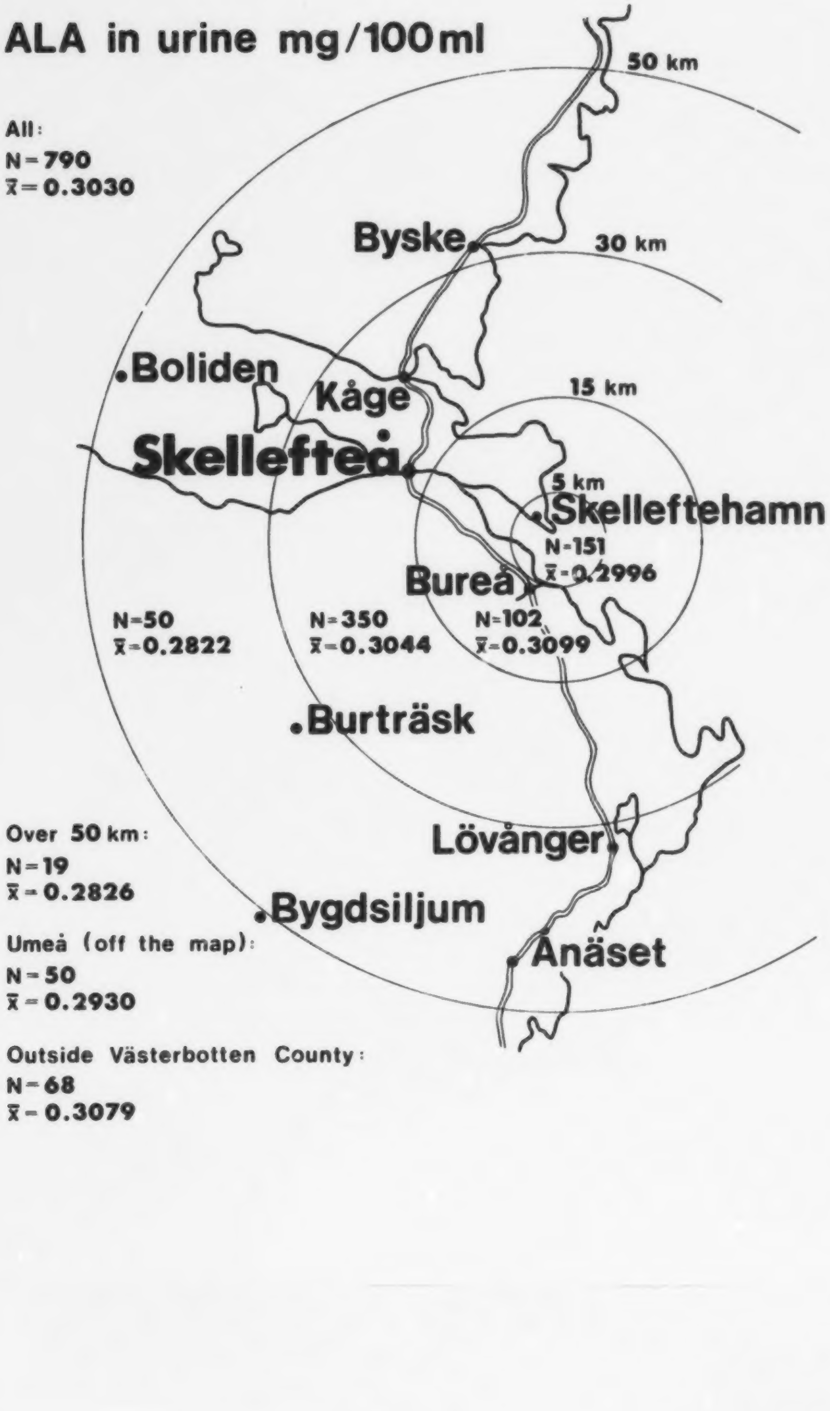
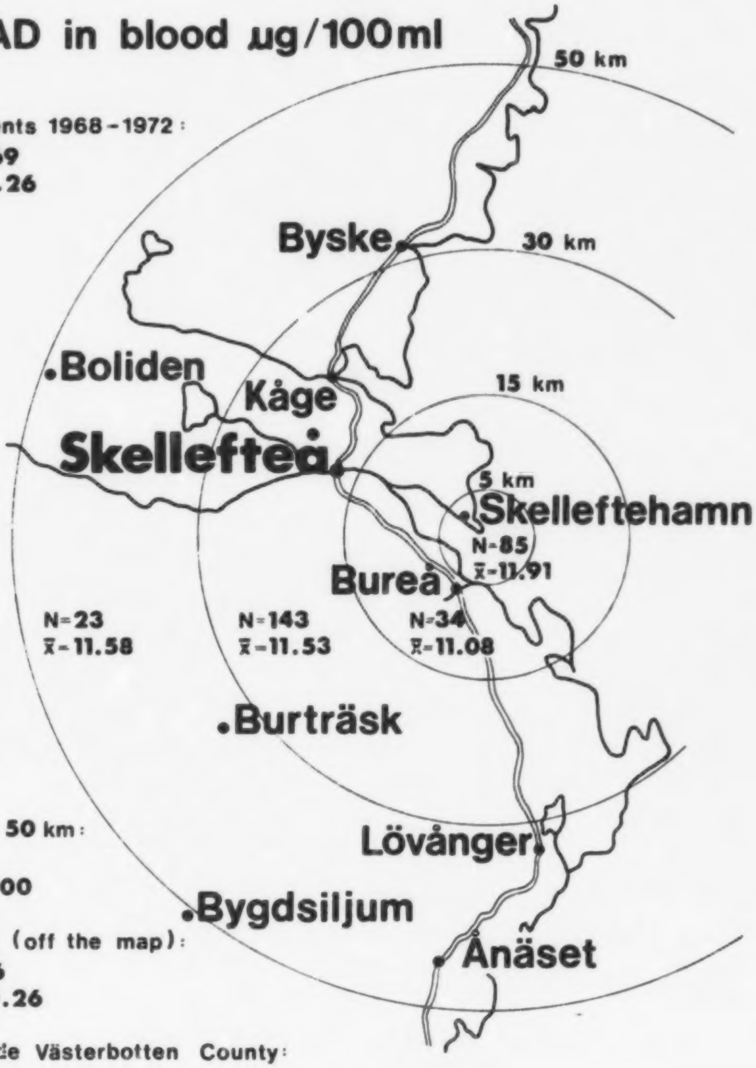


Fig 3

NEW EMPLOYEES

LEAD in blood µg/100ml

Students 1968 - 1972:
N = 369
$\bar{x}$ = 11.26



Over 50 km:
N = 6
$\bar{x}$ = 8.00

Umeå (off the map):
N = 46
$\bar{x}$ = 10.26

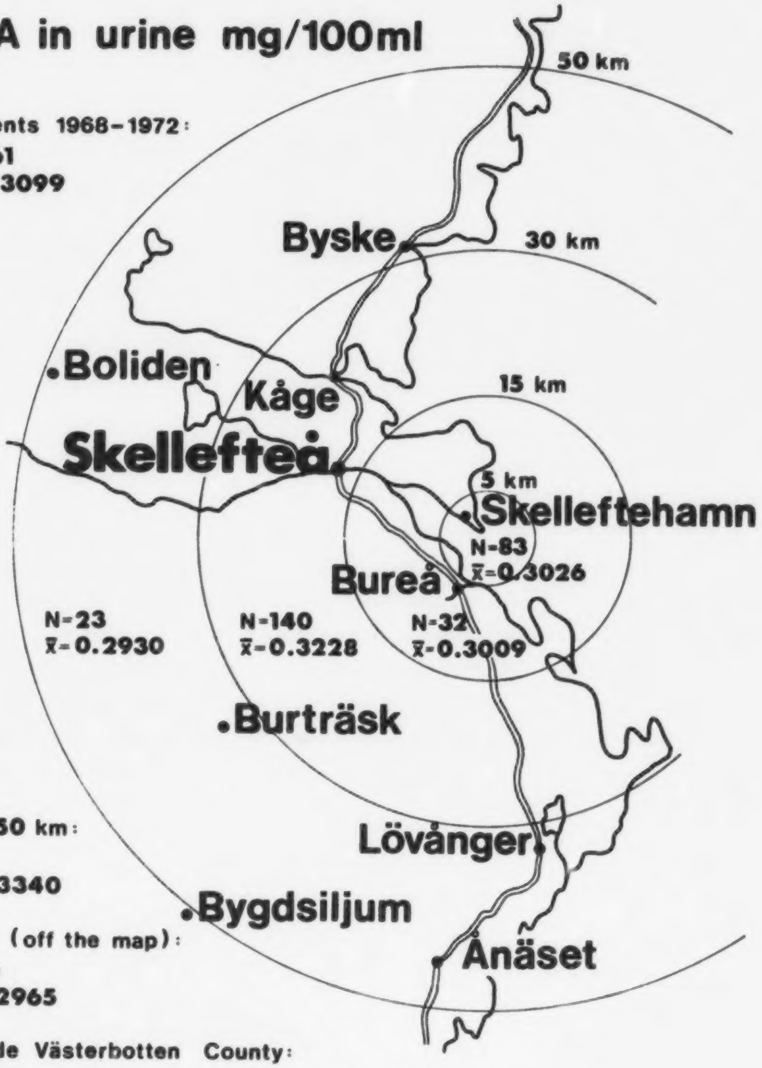
Outside Västerbotten County:
N = 32
$\bar{x}$ = 10.31

Fig 4

NEW EMPLOYEES

ALA in urine mg/100ml

Students 1968–1972:
N = 361
$\bar{x} = 0.3099$



Over 50 km:
N = 5
$\bar{x} = 0.3340$

Umeå (off the map):
N = 46
$\bar{x} = 0.2965$

Outside Västerbotten County:
N = 32
$\bar{x} = 0.3000$

plant. (Fig. 4).

Table 4

Lead level (μg/100 ml) in the blood of workers belonging to different professions

Profession	n	Range	$\bar{X}$	S_D
Students	369	4-40	11.26	5.2
Drivers	33	6-32	13.18	4.8
Electricians	7	6-23	16.14	-
Metal workers	70	8-36	15.94	6.3
Lightweight cement workers, Siporex	20	7-45	14.60	9.1
Wood and pulp workers	39	4-36	11.94	5.9
Other professions	263	4-62	13.05	5.9

It can be seen from Table 4 that the professional group which had the highest level was electricians. But this was a small group (7 persons) and thus not significant. The metal workers had the highest significant mean value, 15.34 μg/100 ml. Metal workers living less than 5 km from the source of emissions exhibited higher values (average 19.06) than metal workers in the other regions. The greatest difference between professions was between electricians and students - 4.88 μg/100 ml. If we disregard students and electricians, the biggest difference between working men was that between metal workers and wood and pulp workers, which amounted to 4.10 μg/100 ml. Thus, profession is more important for the level of lead in the blood than distance of abode from the smelting plant. The largest difference was between metal workers living within 5 km of the plant and students in Umeå - 8.80 μg/100 ml.

There was no difference in the ALA values between these two groups. In fact, there was no difference in the ALA values between any of the professional groups. Thus, we can conclude that there were no differences in metabolic response, even though the lead values were somewhat different (Table 5).

Table 5
ALA in the urine (mg/100 ml) of workers belonging to different professions

Profession	n	Range	$\bar{x}$
Students	361	0.05-2.08	0.3099
Drivers	33	0.09-0.80	0.2948
Electricians	7	0.24-0.57	0.3557
Metal workers	69	0.07-0.64	0.3024
Lightweight cement workers, Siporex	19	0.09-0.50	0.2778
Wood and pulp workers	39	0.09-0.87	0.3064
Other professions	262	0.05-0.73	0.2946

As further proof that occupation has some effect on lead level in the blood, it has been shown that trade school students, who have some practical experience in their trades during their study years, exhibit significantly higher lead levels in their blood than do other students.

Age does not seem to have any influence on lead level in the blood of lead workers. Exposure is the decisive factor. This opinion is shared by a number of researchers in the field, including Lehnert, who surveyed the problem. The lower lead levels in the blood which are sometimes obtained from older age groups may result from the fact that older persons seek work which involves less physical strain, resulting in less inhalation of lead.

The levels of airborne lead are also of interest in this connection. The measurements which have been made at a distance of <5 km from the smelting plant in Skelleftehamn have given results which vary in the actual residential area from 0.3 to 0.5 $\mu g/m^3$ air. Other values measured within the region <5 km from the source of emissions varied between 0.2 and 3.8 $\mu g/m^3$, with a mean of 0.85 $\mu g/m^3$. These data may be compared with the values which were reported at a heavy metals conference in February of 1973 in Düsseldorf. It was for example reported that a lead level in the air of 3 $\mu g/m^3$ could result in a lead level in the blood of 30 μg/100 ml. At a meeting which was held after the conference, a committee of experts decided that an air level of 2 $\mu g/m^3$ could be recommended as acceptable.

Finally, some experiments which shed light upon the relationship between inhaled lead and blood lead levels deserve mention here.

Exposure trials with airborne, inhaled lead, which have been conducted on prisoners in the USA, have shown that when the prisoners were allowed to reside in an atmosphere containing 3.2 μg of lead per m^3 for 125 days, the mean level of lead in the blood rose from about 18 to 27 μg/100 ml. The mean lead level of a group of subjects who were exposed to air containing 10.9 μg lead per m^3 for 125 days rose from about 20 to about 38 μg/100 ml. The control group exhibited no rise. One of the graphs of these experiments presented by Knelson at the heavy metals conference in Düsseldorf is reproduced in Fig. 5. From these experiments, we can conclude that the mean value of 14 μg/100 ml we obtained for all the subjects in our study and 19 μg/100 ml for the metal workers living less than 5 km from the source of emissions indicate a low mean exposure to airborne, inhaleable lead, in any case less than 3.2 $\mu g/m^3$ and probably much lower.

The lead levels in the Skelleftehamn air are low, probably much lower than the guideline value of 2 $\mu g/m^3$ offered in Düsseldorf. This claim is supported by measurements of the lead content of the air, the value found for the mean level of lead in the blood of men in Skelleftehamn and finally the American lead exposure experiments on prisoners discussed above.

In summary, we can conclude that the lead emissions from the Rönnskär Works have not proved to constitute a health hazard for people living in the surrounding area.

DISCUSSION

BERLIN (C.E.C.)

1. Could the author indicate the analytical method used for ALA determinations and how the quality was carried out?

2. Has any assessment been made of the emissions from the smelter and of environmental concentrations, besides air?

HOLMQVIST (Sweden)

1. The ALA determinations were made according to a method elaborated by Mauzerall and Granick (1956). The quality control was carried out by several investigations. The recovery from normal human urine was satisfactory. Comparison with other laboratories gave comparable results.

2. The emissions from the smelter have continuously been reduced during the last decades. The measures for improvements of the environment have been intensified during the last years, among other things by new electrofilters and by building a plant for production of liquid sulphur dioxide from the smelter gases.

STUDY ON CADMIUM PROTEINURIA. GLOMERULAR DYSFUNCTION: AN EARLY SIGN OF RENAL IMPAIRMENT

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ABSTRACT

The electrophoretic patterns of urinary proteins from workers with prolonged exposure to cadmium, reveal the existence of two distinct proteinurias, i.e., glomerular and mixed. The former probably reflects only an enhanced permeability of the glomeruli to high molecular weight plasma proteins, whereas in the latter also a decreased protein reabsorption by the tubuli is involved. Glomerular patterns were found in workers with less and more than 20 years exposure; mixed patterns only in workers with more than 20 years exposure. These observations were confirmed by measurements of renal clearance of albumin, lysozyme, and ribonuclease. The results indicate that in workers with a glomerular pattern only an increased excretion of high molecular weight plasma proteins occurs, but that in those with a mixed pattern the excretion of both high and low molecular weight plasma proteins is increased. These results together with a few recent reports on animals exposed to Cd and on electron microscopic studies in patients suffering from Balkan endemic nephropathy, reinforce our suggestion that a glomerular dysfunction may precede the "classical" tubulopathy observed after long-term exposure to cadmium.

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Introduction

Although the complex handling of plasma proteins in the kidneys is presently not fully understood, the types and relative amount of plasma proteins excreted in urine are useful biological parameters reflecting renal function. The predominant part of plasma proteins in urine appears to arise from a process of glomerular filtration followed by reabsorption and catabolism in the proximal tubuli (Schultze and Heremans (1).

Cadmium is a systemic cumulative poison frequently inducing increased proteinuria in workers with long exposure to cadmium compounds as first described by Friberg (2). This finding has been amply confirmed by results of epidemiological surveys among workers chronically exposed to cadmium oxide dust or fume and by studies with experimentally poisoned animals, revealing that the injurious action of Cd occurs especially in the proximal tubuli with a concomitantly elevated excretion of low molecular weight proteins (for reviews see: Flick et al. (3) ; Friberg et al. (4) among them the plasma protein. β_2-microglobulin (Piscator (5) ; Berggård and Bearn (6).

Up to now, most reports on cadmium proteinuria have dealt with workers excreting large amounts of proteins (.5g or more a day) and because of the obvious tubular lesion, slight changes in glomerular permeability could have been overlooked. Recent investigation in our laboratory, however, prompted us to suggest that glomerular dysfunction may be an early phase in cadmium-induced renal impairment (Lauwerys et al. (7). Indeed, electrophoresis on agarose of the urinary proteins of clinically healthy workers, exposed to an average airborne concentration of Cd-dust below the current American TLV (0.2 mg/m^3 of air), enabled us to classify the proteinuria in two categories, i.e., glomerular and mixed (glomerular + tubular) proteinuria. For both types of proteinuria the electrophoretic patterns reflect an enhanced excretion of high molecular weight proteins (i.e., albumin,α_2-Zn-andα_2-HSglycoprotein, transferring), and in cases of mixed proteinuria also an enhanced excretion of low molecular weight proteins (i.e., α_2-and β_2-microglobulin, and in some cases post-γ- protein). The glomerular type was observed in workers with less or more than 20 years' occupational exposure, whereas the mixed type only in workers with more than 20 years' exposure.

The present paper reports results of investigations carried out to test our hypothesis that early glomerular dysfunction may be induced by cadmium. We have estimated the relative importance of glomerular and tubular proteinuria by measuring the renal clearances of albumin, lysozyme, and ribonuclease (expressed as % of the (creatinine clearance) in both distinct "electrophoretic groups" of Cd-exposed workers.

Materials and Methods

The total study population consisted of 218 male workers (110 non-exposed and 108 exposed) from two different cadmium-producing plants and a Ni-Cd storage battery factory in Belgium. In each factory the selected group of non-exposed workers matches the group of exposed workers according to age, weight, height, smoking habit, duration of employment in the same factory, and socio-economic status. Air sampling for determination of total airborne and respirable (aerodynamic diameter $< 5\ \mu$) Cd-dust concentration at different sites of the factories was carried out as described previously (7). Cadmium was measured by atomic absorption spectrophotometry.

The following biological analyses were performed: Cd concentration in blood and urine (Vens and Lauwerys 8), total proteinuria(7), total α-amino-aciduria (Hall et al. 9) and electrophoresis on agarose of urinary proteins (Johansson 10 ; see also 7). Furthermore, the concentration of lysozyme and ribonuclease (Harrison et al. 11), albumin(Ritchie et al. 12), and creatinine (Jaffe-method, see Henry 13)were measured on the urine and blood specimen (collected at the same time as the urine samples of 6 normal subjects (laboratory staff),18 Cd-exposed workers with pathological electrophoretic pattern and 8 patients with various renal diseases.

Results

The workers were exposed to an average respirable Cd-dust concentration of 0.020 to 0.030 mg/m^3 of air (average total airborne concentration ranged from 0.074 to 0.212 mg/m^3 of air) and appeared to be in good clinical condition.

Table I shows the biological parameters measured in the total study population; the results obtained for the exposed workers are classified according to the electrophoretic pattern of the urinary proteins, i.e., normal, glomerular and mixed. All the non-exposed workers showed normal electrophoretic pattern, while 18 out of 108 exposed workers exhibited a pathological one.

As reported previously (7) the glomerular pattern was found in workers with less (4 cases) or more (5 cases) than 20 years' exposure, whereas the mixed (9 cases) only in those with more than 20 years' exposure. The mean Cd concentration in urine (Cd-U) of non-exposed workers is about five times that (0.38 ± 0.10 μg/g creatinine) found in 20 normal male subjects (students and laboratory staff; non-smokers). This discrepancy probably reflects a different degree of environmental contamination by Cd between the living areas of the normal subjects and those of the non-exposed workers which are likely to be more contaminated because of the proximity of the Cd plants.

Ninety exposed workers showed apparently normal kidney function at least on the basis of the electrophoretic pattern of urinary proteins, total proteinuria, and total α-amino-aciduria (Table I). For 9 exposed workers a glomerular pattern was found on the urinary protein electrophoresis. They also exhibited a significant increased total proteinuria and a slight increase in total α-amino-aciduria. It is interesting to notice that the average Cd-U level of these workers (19.0 μm/g creatinine) was not different from that of the exposed group (18.7 μg/g creatinine) with normal electrophoretic urinary protein pattern. The 9 other exposed workers showed a more impaired renal function as evidenced by the finding

Table I Biological parameters in workers non-exposed to cadmium and in exposed workers classified according to the electrophoretic pattern of their urinary proteins (mean values ± SEM)

Workers	Electrophoreti pattern	N	Cd in urine (µg/g creatinine)	Proteinuria (mg/g creatinine)	α-Aminoaciduria (mg/g creatinine)	Cd in blood (µg %)
Non-exposed	Normal	110	1.79 ± 0.16*	152 ± 5.0	159 ± 6.1	0.58 ± 0.02
Exposed	Normal	90	18.7 ± 2.3	166 ± 14	158 ± 5.9	2.18 ± 0.19
	Glomerular	9	19.0 ± 3.7	296 ± 47	175 ± 10	1.87 ± 0.38
	Mixed	9	52.8 ± 8.3	700 ± 131	232 ± 25	2.72 ± 0.55

*Significant different mean values (P at least < 0.05 ; Student's t-test).

Table II Renal clearance of three plasma proteins in normal subjects, in Cd-exposed workers with abnormal electrophoretic pattern (glomerular and mixed), and in patients with renal diseases (mean and range)

Subjects	Renal clearance of plasma proteins (as % of endogenous creatinine clearance)			Proteinuria (mg/g creatinine)	Creatinine in serum (mg/100 ml)
	Albumin (10^{-4})	Lysozyme	Ribonuclease		
Normal subjects (n = 6)	0.35	0.010	5.31	161	1.18
	0.11-0.70	0.006-0.019	2.64-9.06	109-228	0.97-1.41
Cd-exposed workers					
Glomerular type (n = 9)	4.05	0.014	5.59	296	1.20
	0.76-16.4	0.005-0.025	1.18-18.5	141-487	0.95-1.84
Mixed type (n = 9)	8.81	2.45	8.73	700	1.28
	0.89-23.3	0.13-20.7	4.19-31.5	267-1171	0.89-2.03
Patients with renal diseases					
Suspected lithiasis	0.58	0.004	0.62		0.97
Uric acid nephrolithiasis	1.13	0.001	0.74		0.99
Focal glomerulonephritis	0.83	0.001	0.70		0.83
Interstitial nephritis (analgesic)	1.68	0.027	0.71		1.61
Interstitial nephritis (analgesic)	281	0.084	5.26		2.01
Acute interstitial nephritis	18.9	0.037	1.84		2.24
Diabetic nephropathy	42.4	0.063	1.30		2.63
Chronic pyelonephritis	263	0.836	17.8		8.69

of a mixed electrophoretic pattern of urinary proteins, a greater increase in total proteinuria and in total α-aminoaciduria. In the last group Cd-U is significantly greater than in the two preceding groups which is a usual observation when the proximal tubuli are damaged by cadmium (7); Adams et al. (14); Kazantzis et al. (15).

Table II shows the results of renal clearance measurements in 6 normal subjects, in the Cd-exposed workers with pathological electrophoretic pattern of urinary proteins (9 glomerular and 9 mixed), and in a group of patients with various renal diseases. For the normal subjects the clearances of albumin (mol. wt. 69 000), lysozyme (mol.wt. 14 000), and ribonuclease (mol. wt. 13 000) expressed as % of the endogenous creatinine clearance, are comparable with literature data (Harrison et al. (11); Peterson et al. (16).

The clearance results obtained for the 18 Cd-exposed workers are in agreement with our classification of the type of proteinuria based on the electrophoretic pattern. Indeed, the moderate increase in proteinuria observed for the 9 workers with glomerular type electrophoretic pattern can only be ascribed to an increased clearance of albumin and of other high molecular weight proteins, since no increased lysozyme and ribonuclease clearances were observed. On the other hand, in the 9 workers with mixed type electrophoretic pattern, not only albumin clearance was increased but also that of low molecular weight proteins as shown by the results of lysozyme and ribonuclease and clearance measurements. The group of patients with renal diseases has been included in this study in order to check the validity of the methods used for measuring the clearances.

Discussion

Results of the renal clearance of high and low molecular weight plasma proteins tend to confirm our previous suggestion (7), that the early kidney lesion in workers developing cadmium-induced proteinuria could be a glomerular dysfunction, which precedes the tubulopathy predominantly found in workers with prolonged exposure to cadmium and excreting large amounts of proteins (14;15) Piscator (17)). Two recent reports dealing with investigation of experimentally Cd-poisoned animals reinforce this suggestion: 1) stop-flow analysis performed on rabbits at the end of successive Cd administration revealed a glomerular dysfunction and a normal or slightly altered proximal tubular function (Nomiyama et al.(18) and (2) long-term (120 days) administration of Ca-adequate and Ca-deficient diets together with water containing 50 ppm Cd provoqued extensive glomerular as well as tubular lesions in male rats (Itokawa et al.(19)). Recently, it has been demonstrated by means of electron microscopy. that in patients with suspected Balkan endemic nephropathy (20) early glomerular lesions are involved first in this renal disease that eventually gives rise to a proteinuria that resembles (Hall et al. (21)). the "classical" tubular proteinuria observed in workers with prolonged exposure to cadmium.

Heinemann et al. (22) (see also Flynn and Platt (23) proposed two basic mechanisms which can cause pathological excretion of high molecular weight proteins: 1) increased glomerular permeability to high molecular weight plasma proteins and no change in tubular reabsorption of protein (only rise of high molecular weight proteins in urine), 2) normal glomerular filtration of high molecular weight proteins with impaired tubular reabsorption which is usually associated with an increased excretion of both high and low molecular weight protein

According to this schema, two hypotheses remain to explain the glomerular type electrophoretic pattern associated with an increased albumin clearance found in 9 workers exposed to cadmium: 1) an impaired glomerular permeability with a normal tubular function or 2) a selective impairment of the mechanism of high molecular weight protein reabsorption by the tubuli with a normal glomerular function. In this last event, the mixed type electrophoretic pattern would then result from an aggravation of the tubular lesion involving also the mechanism of low molecular weight protein reabsorption.

However, the morphologic studies (19, 20) mentioned above tend to support the first hypothesis, i.e., that an alteration of glomerular filtration is responsible for the glomerular type electrophoretic pattern, and that the mixed pattern results from lesion at both sites (glomeruli and tubuli) usually occurring after a much longer exposure to cadmium than the glomerular lesion.

Measurement of β_2-microglobulin clearance will be carried out to characterize further the two types of proteinuria.

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UNTERSUCHUNG DER WIRKUNGEN AUF DEN MENSCHEN
HUMAN EFFECTS STUDIES
ETUDES DES EFFETS SUR L'HOMME
STUDI DEGLI EFFETTI SULL'UOMO
ONDERZOEKINGEN NAAR EFFECTEN BIJ DE MENS

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INCREASED RISK OF ACUTE AND CHRONIC RESPIRATORY DISORDERS FOLLOWING LONG-TERM AIR POLLUTION EXPOSURES

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ABSTRACT

In 1970 and 1971, the U.S. Environmental Protection Agency conducted several epidemiologic surveys which related long-term air pollution exposures to the prevalence of chronic respiratory disease and the incidence of acute respiratory disease. Chronic respiratory disease (CRD) was studied in about 19,000 young adults in New York, Utah and the Idaho-Montana area, and in about 42,000 military inductees in Chicago. Acute respiratory disease (ARD) was studied in about 5,500 children and adults in the cities of Chicago and New York.

In all four CRD studies, chronic bronchitis prevalence was elevated in neighbourhoods of elevated pollution exposure. The effect of pollution on chronic bronchitis prevalence generally ranged from about one-tenth to about one-third of the effect of cigarette smoking. Among fathers in New York, the pollution effect on CRD was observed to be stronger than the smoking effect. The latter finding emphasized the considerable effect of air pollution on CRD prevalence.

The findings of the Chicago and New York ARD studies suggested that reduced rates of ARD would result from improvements in existing air quality. In Chicago, it was estimated that reduction of annual average sulfur dioxide (SO_2) levels from about 100 $\mu g/m^3$ to about 57 $\mu g/m^3$, and of suspended sulfate (SS) levels

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from about 18 $\mu g/m^3$ to about 14 $\mu g/m^3$, would promote reductions in total ARD incidence. Such reductions in ARD could be expected even in the face of continued exposure to total suspended particulate levels of about 110 to 150 $\mu g/m^3$. In New York, exposure for one to three years to annual average SO_2 levels of about 250 $\mu g/m^3$, accompanied by SS levels of about 22 $\mu g/m^3$ and TSP levels of about 94-117 $\mu g/m^3$, was associated with a significant increase in the incidence of acute lower respiratory illness.

1. Introduction

The U.S. Environmental Protection Agency (EPA) has devoted considerable effort to characterizing the health effects of long-term past exposures to ambient air pollution. The purpose of this report is to summarize four EPA determinations of pollution effects on the prevalence of chronic respiratory disease (CRD)[1,2,3,4,5], and two determinations of pollution effects on the incidence of acute respiratory disease (ARD).[6,7,8] The CRD studies focused on young adults, and the ARD studies focused on both young adults and their children. For all six studies, the pollutants of major concern were oxides of sulfur and suspended particulate matter (TSP). One important pollutant, suspended sulfates, fits into both of these categories.

2. Methods

2.1 Determinations of CRD Prevalence

In 1969 and 1970, the prevalence of CRD was determined in four large geographic areas of the United States, the Salt Lake Basin in Utah, the two-state Idaho-Montana area, the Chicago, Illinois area, and the New York City Metropolitan area. The first two areas are in the western United States, and the bulk of their sulfur oxide and particulate pollution is emitted by metal smelters. The pollution in Utah and Idaho-Montana generally contains substantial levels of sulfur oxides, including suspended sulfates, but moderate levels of TSP. In Chicago and New York, substantial amounts of both sulfur oxides and TSP are present.

In each of these four major areas, at least one community or zone of high pollution exposure, and at least one community or zone of low exposure were selected for study. Thus, four independent comparisons of CRD rates in the presence of high and low pollution levels could be made. The results of each individual CRD study therefore offered a test of the results of the other three.

In Utah, Idaho-Montana, and New York, CRD prevalence rates were determined by the distribution of self-administered symptom questionnaires to about 1400 families of elementary school children in each community. (In Utah, questionnaires were also distributed to families of high school students.) Questionnaires were patterned after the 1966 British Medical Research Council's Questionnaire on Research into Chronic Bronchitis. In each community, over 1000 completed questionnaires were returned for data processing. Response rates ranged between 85 to 95 %. In Chicago, similar self-administered questionnaires were distributed to 38,800 white and 3200 black male military inductees between 18 and 24 years of age. Study participants who reported coughing and phlegm from the chest for at least three months per year were considered to have chronic bronchitis.

2.2 Determinations of ARD Incidence

Determinations of incidence rates of ARD were made in Chicago from December 1969 through November 1970, and in New York from September 1970 until May 1971. As with CRD, determinations of ARD rates were made across pollution gradients in both areas. In each area, participating families were enrolled in a biweekly telephone survey, in which trained interviewers inquired about the presence of acute upper and lower respiratory complaints in all family members during the previous two weeks. In both areas, incidence rates were computed for total ARD, for lower respiratory disease, and for upper respiratory disease.

For both Chicago and New York, ARD rates were computed for four family segments, mothers, fathers, elementary school children (aged 5-12 years), and preschool children (aged less than 5 years). In Chicago, about 625 families, representing about 2700 individuals, participated in the ARD survey. In New York, about 650 families, representing about 3000 individuals, participated. All ARD study participants in Chicago and New York lived within 1 1/2 miles of a municipal air monitoring station.

2.3 Exposures to Air Pollution

For all communities or zones in the four study areas of Utah, Idaho-Montana, Chicago, and New York, estimates of past air pollution were made for each year of the 20 to 30 years preceding the CRD survey. Three major tools were used to construct these estimates; actual aerometric measurements made by local, state, or federal organizations; emissions data from smelters and other industrial complexes; and meteorologic models of pollution dispersion. Aerometric data for the periods of the ARD survey were provided by the cities of Chicago and New York.

3. Results

3.1 CRD Prevalence Surveys

In each of the four study areas, levels of sulfur oxide and particulate pollution tended to decline over the 20 years preceding the CRD prevalence survey in that area (Table 1). The pollutant showing the most dramatic decreases was SO_2. Declines in TSP, though quite steady, were not proportionally as large as those in SO_2. Levels of SS were generally estimated to have undergone the smallest proportional decrements. (An exception to these trends occurred in Idaho-Montana, where the estimates of maximum levels of SO_2 and SS actually increased slightly between 1950-59 and 1968-70).

In all four study areas, communities were divided into high-exposure and low-exposure categories on the basis of estimated exposures during the 20 to 30 years preceding the CRD surveys. Within each of the high-exposure and low-exposure categories, chronic bronchitis prevalence rates were computed separately for mothers and fathers. (In Chicago, rates were computed separately for black and white inductees.) Within these categories, rates were computed separately for lifetime non-smokers, ex-smokers, and current cigarette smokers.

With the exception of black military inductees from low-pollution areas around Chicago, all groups reported higher chronic bronchitis prevalence among smokers than among non-smokers (Table 2). Prevalence rates among ex-smokers were generally intermediate between non-smokers' and current smokers' rates. In both non-smokers and current smokers, prevalence rates were consistently higher in areas of elevated air pollution than in those of low pollution. In all four areas, the highest prevalence rates were found in smokers living in communities or zones of elevated pollution.

An effort was made to determine the effect of air pollution relative to cigarette smoking on chronic bronchitis prevalence rates. The first step in this determination was to calculate the excess prevalences due to smoking alone and to pollution alone. In each area-specific and sex-specific group, the excess prevalence attributable to smoking is equal to the difference between the prevalence among smokers in the low-pollution communities and the prevalence among non-smokers in the low-pollution communities. The excess prevalence attributable to pollution is equal to the difference between the prevalence among

TABLE 1

Range of Exposure to Air Pollutants (in µg/m^3)
In Four United States Areas, During 1950-1959
and During 1968-1970

Time Period	Range of Exposure (in µg/m^3)					
	SO_2		TSP		SS	
	Minimum (Area)*	Maximum (Area)	Minimum (Area)	Maximum (Area)	Minimum (Area)	Maximum (Area)
1950-59	142 (U)	419 (NY)	139 (I-M)	214 (C)	15 (I-M)	27 (NY)
1968-70	92 (U)	210 (NY)	88 (U)	155 (C)	15 (U)	20 (NY)

*Area abbreviations are as follows:

U = Utah
I-M = Idaho-Montana
C = Chicago
NY = New York

TABLE 2

Chronic Bronchitis Prevalence Rates, Distributed by Smoking Status and Pollution Exposure: United States Studies, 1969-1970

Community Exposure & Smoking Status	Chronic Bronchitis Prevalence (in %)							
	Utah		Idaho-Montana		New York		Chicago	
	Mothers	Fathers	Mothers	Fathers	Mothers	Fathers	Blacks	Whites
Low Pollution								
Nonsmokers	4.16	3.00	1.08	1.25	2.00	4.60	9.20	4.00
Smokers	15.80	19.60	11.78	17.05	13.90	13.89	8.74	15.20
High Pollution								
Nonsmokers	5.20	6.81	2.54	3.48	6.04	15.87	9.84	5.32
Smokers	22.25	26.80	12.88	18.36	18.08	21.71	13.52	18.08

non smokers in the high-pollution communities and the prevalence among non-smokers in the low-pollution communities. (For black inductees in Chicago, the excess prevalence due to smoking was calculated to be a negative number. As yet, we have no convincing explanation for this unusual finding.)

The second step in this determination was to divide the excess prevalence attributable to pollution by the excess prevalence attributable to smoking. For mothers, the pollution effect on chronic bronchitis prevalence rates was 9% to 34% as strong as the smoking effect (Table 3). For fathers, the pollution effect was 14% to 121% as strong as the smoking effect. For both mothers and fathers, the effect of pollution relative to smoking was strongest in New York. In white military inductees, the pollution effect was 18% as strong as the smoking effect.

2. ARD Incidence Surveys

2.1 Chicago Study

In Chicago, ARD study participants were divided into a high and a low air pollution exposure group. Mean pollution exposures for both groups during the study period are presented in Table 4. In all family segments in Chicago, the incidence rates of upper and lower tract ARD combined were higher in the high-exposure group than in the low-exposure group (Table 5). Increases in total ARD between exposure groups ranged from 2% in children aged 3 to 5 years, to 37% in children aged less than three years. Increases in rates between exposure groups were particularly apparent in families who had lived at the same address for at least three years. In Chicago, differences between exposure groups in rates of both upper and lower tract ARD were statistically significant at $\alpha = 0.05$.

2.2 New York Study

In New York, ARD incidence rates in the community of lowest air pollution exposure were compared to the combined rates in the two communities of higher exposure. Mean pollution exposures for the high- and low-exposure groups during the study period are presented in Table 4. Incidence rates of total ARD in New York, as in Chicago, were generally higher in the high-exposure communities than in the low-exposure communities. Elevations in rates between exposure areas were statistically significant for lower, but not for upper respiratory

TABLE 3

Excess Chronic Bronchitis Prevalence Attributable
to Cigarette Smoking and to Air Pollution, United States Studies,
1969-1970

Source of Risk	Excess Chronic Bronchitis Prevalence (in %)							
	Utah		Idaho-Montana		New York		Chicago	
	Mothers	Fathers	Mothers	Fathers	Mothers	Fathers	Blacks	Whites
Pollution	1.04	3.81	1.46	2.23	4.04	11.27	0.64	1.32
Smoking	11.64	16.60	10.70	15.80	11.90	9.29	-0.46	11.20
Pollution/Smoking	0.09	0.23	0.14	0.14	0.34	1.21	-1.39	0.18

TABLE 4

Mean Pollution Exposures, in µg/m^3, During
Acute Respiratory Disease Surveys in Chicago (1969 - 1970) and
New York (1970-71)

Area	Mean Pollution Level (in µg/m^3)					
	SO_2		TSP		SS	
	Low Exposure	High Exposure	Low Exposure	High Exposure	Low Exposure	High Exposure
Chicago	57	106	111	151	14.5	16.0
New York	23	63	34	104	10.2	14.3

TABLE 5

CHICAGO: RELATIVE RISK OF TOTAL ACUTE
RESPIRATORY DISEASE

FAMILY SEGMENT	COMMUNITY AIR POLLUTION EXPOSURE		
	INTERMEDIATE		HIGHEST
FATHERS	1.00	(2.80)*	1.33
MOTHERS	1.00	(4.76)	1.25
SCHOOL CHILDREN	1.00	(7.04)	1.18
CHILDREN 3-5	1.00	(9.35)	1.02
CHILDREN 0-2	1.00	(9.41)	1.37

* (BASELINE ARD RATES, PER 100 PERSON-WEEKS, ARE IN PARENTHESES.)

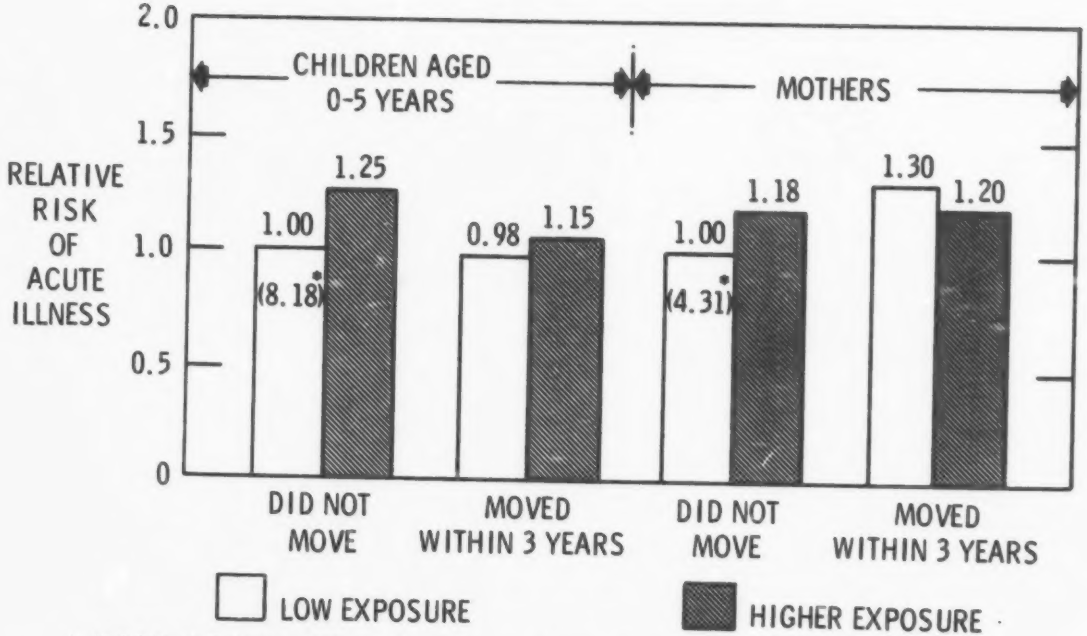
disease. Elevations in rates were more apparent in people who had not changed address in the past three years than in those who had (Figure 1).

4. Discussion

The CRD prevalence surveys consistently demonstrated higher chronic bronchitis rates in polluted communities than in relatively clean ones The effect of pollution relative to smoking, which ranged from 9% to over 100%, was generally stronger than the investigators had expected. From the CRD survey results, we would not argue that air pollution is as strong a determinant of chronic bronchitis as is heavy smoking. We would suggest, however, that long-term exposure to sulfur oxide and particulate pollution combined may rival moderate smoking in promoting the development of that disease.

In the ARD incidence surveys, illness rates were consistently highest in residentially stable families in high-pollution neighborhoods. This result suggested that several years of continuous exposure to elevated pollution levels might be required to develop increased susceptibility to ARD. The ARD survey results also suggested that improvements in air quality, such as have occurred in many American cities since 1970, might be accompanied by decreased ARD rates. In the investigators' best judgment, reductions in annual average SO_2 levels from about 110 $\mu g/m^3$ to about 57 $\mu g/m^3$, accompanied by reductions of SS from about 18 to about 14 $\mu g/m^3$, might well promote reductions in the incidence of acute respiratory disease.

FIGURE 1



* (BASELINE ARD RATES, PER 100 PERSON-WEEKS, ARE IN PARENTHESES.)

FIGURE 1: NEW YORK: EFFECT OF MOBILITY ON TOTAL ACUTE RESPIRATORY DISEASE, 1970-1971

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DISCUSSION

SULAIMAN (Nigeria)

In Nigeria where 10-15% of people carry sickle cell trait or sickle cell anaemia, what part has carbon monoxide to play in causing adverse human effects?

CHAPMAN (U.S.A.)

To my knowledge, very little research is available on the effects of carbon monoxide on patients with sickle cell anaemia. In people without that disease, levels of about 50 ppm carbon monoxide have been well demonstrated to shorten the time required for exercise to produce angina pain in cardiac patients.

FREEMAN (U.S.A.)

Do you happen to have equivalent data with regard to either NO_2 or O_3 or both?

CHAPMAN (U.S.A.)

Within the next six months, EPA reports will be written on the effects of nitrogen oxides and oxidants on chronic respiratory disease and other indicators.

HICKEY (Ireland)

What is your definition of a smoker? Have you distinguished between smoking habits of the mothers and the fathers?

CHAPMAN (U.S.A.)

In this report, I have defined a smoker as anyone who currently smokes cigarettes. Generally, we have observed that smoking rates are higher in fathers than in mothers, and that chronic bronchitis rates are highest in fathers who smoke. However, we have also observed that chronic bronchitis are higher in non-smoking fathers than in non-smoking mothers. This last observation was particularly striking in polluted communities in New York, where non-smoking mothers had a chronic bronchitis rate of 6.04%, and non-smoking fathers had a rate of 15.87%.

BRONCHITE CHRONIQUE, SYMPTOMES RESPIRATOIRES ET POLLUTION ATMOSPHERIQUE

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RESUME

Dans cette étude, on a d'une part appliqué le questionnaire sur la bronchite chronique du British Medical Council à 2882 personnes appartenant à la population active du Canton de Genève et d'autre part analysé les données de pollution atmosphérique enregistrées à 10 postes situés en ville ou à la campagne. Les teneurs en SO_2 *rencontrées (teneur moyenne:* $30\ \mu g/m^3$ *en ville) dépendent essentiellement des chauffages domestiques, la pollution industrielle étant pratiquement négligeable. Pour définir le niveau de pollution moyen durant la période de l'enquête, on a tout d'abord éliminé, à l'aide d'un programme de désaisonnalisation, les variations saisonnières, puis cherché, à l'aide d'une régression multiple par pas, une relation entre les niveaux moyens aux 10 postes et 2 paramètres exprimant l'abondance des sources de pollution par le* SO_2*, soit la densité de population et le chiffre d'affaires théorique des ramoneurs. Une carte par commune des niveaux de pollution a ainsi pu être établie où l'on a placé les prévalences de la bronchite chronique ou des symptômes respiratoires non bronchitiques strictu senso. On a enfin introduit un niveau personnel de pollution par individu en tenant compte du lieu d'habitation.*

D'après les résultats obtenus jusqu'ici et en utilisant le SO_2 *comme indicateur, la prévalence de la bronchite chronique tend à augmenter avec l'augmentation de la pollution provenant*

des chauffages. Cette relation devient hautement significative pour autant que l'on puisse recourir à plusieurs indices de la pollution. Il n'est pas exclu que la pollution atmosphérique telle qu'elle existe à Genève contribue aussi à aggraver les symptômes respiratoires qu'ils soient spécifiques ou non.

ABSTRACT

In this study the British Medical Council questionnaire on chronic bronchitis was used to obtain information on 2882 people belonging to the working population of the Canton of Geneva; secondly, atmospheric pollution data recorded at ten points in urban and rural areas were analysed. The SO_2 concentrations encountered (average concentration 30 $\mu g/m^3$ in town) originated principally from domestic heating, pollution of industrial origin being practically negligible. To define the average pollution level during the survey period we first of all eliminated seasonal variations by a "deseasonalisation" process and then, by applying multiple step-by-step regression, sought a relationship between the average levels at the ten control points and two parameters expressing the frequency of SO_2 pollution sources, or the population density and the theoretical volume of business of chimney-sweeps. Maps showing pollution levels could thus be drawn for each commune where chronic bronchitis or respiratory symptoms not strictly speaking bronchitic were shown to be prevalent. Finally, a personal pollution level per individual was included, according to the place of residence.

From the results obtained to date, and using SO_2 as an indicator, it appears that the prevalence of chronic bronchitis tends to increase with the increase of pollution from central heating systems. This connection becomes highly significant as long as it is possible to refer to several pollution indices. The possibility is not excluded that atmospheric pollution, as existing in Geneva, contributes to the aggravation of respiratory symptoms whether specific or not.

INTRODUCTION

La mise en relation de la pollution atmosphérique avec ses effets sur la bronchite chronique a déjà fait l'objet de nombreux travaux dont quelques uns ont été revus dans un article précédent (1). Actuellement encore, de larges enquêtes sont menées sur ce sujet, attestant par là qu'il est loin d'être épuisé. Il présente, en effet, cette difficulté que la pollution atmosphérique entre en concurrence avec d'autres facteurs exogènes comme la fumée du tabac ou l'exposition aux polluants d'origine professionnelle. De manière à distinguer l'action propre de la pollution atmosphérique, on recourt le plus souvent à la sélection de groupes comparables sur plusieurs points habitant par exemple, soit la ville, soit la campagne. On interroge communément les habitants d'un secteur proche d'un point de mesure du polluant mesuré. Ce type d'enquête réclame une population assez dense pour qu'on puisse y trouver les groupes appropriés et une région géographique assez étendue pour que les niveaux de pollution soient très contrastés. Dans notre enquête, la situation de départ est différente : le territoire considéré est exigu (284 km2 pour 332.000 habitants) et le gradient de pollution dans l'espace, faible au départ, a décru ces dix dernières années. C'est pourquoi nous nous sommes orientés vers une autre méthodologie qui, sans nous permettre des conclusions définitives, peut orienter nos recherches futures et éventuellement inspirer d'autres enquêtes menées dans de semblables conditions. Cette méthode est une tentative de mettre en oeuvre des analyses mathématiques où d'une part des phénomènes connus et répertoriés peuvent être utilisés à la place des dosages chimiques de la pollution (qu'on ne saurait multiplier à l'infini), et où d'autre part les facteurs de pollution, introduits dans une série d'autres variables, manifestent leur pouvoir discriminant vis-à-vis des bronchitiques ou des non-bronchitiques du collectif examiné.

METHODOLOGIE ET RESULTATS

1. Pollution atmosphérique : les données

Il s'agit des niveaux relatifs de SO_2 déterminés, en une dizaine de points du Canton de Genève, à l'aide d'appareils Leclerc, par les soins du Laboratoire de toxicologie et d'analyse de l'air (Dir. P. Desbaumes) de l'Institut d'hygiène. Si les appareils Leclerc présentent l'inconvénient de ne pas fournir de concentration, ils ont l'avantage d'être peu coûteux et d'un maniement aisé, ce qui a permis le relevé systématique de valeurs durant plus de 10 ans.

2. Conditions atmosphériques : les données

Il s'agit de relevés faits à l'aéroport de Cointrin, portant sur la température de l'air, son humidité, le point de rosée, la visibilité, la pression atmosphérique, etc. Etant donné l'exiguité du canton, on peut admettre que ces relevés fournissent une bonne approximation des conditions météorologiques qui règnent sur l'ensemble du territoire. La situation géographique très particulière du canton de Genève explique en grande partie comment les conditions météorologiques évoluent dans l'année et comment elles influencent la répartition, sur tout le territoire, des polluants atmosphériques qui proviennent en majorité de l'agglomération urbaine. Le canton de Genève est en effet enfermé entre les barrières naturelles du Jura et des Alpes. Les vents sont ainsi canalisés dans deux directions privilégiées et sont associés à des climats très opposés. Quand le vent du Nord-Est ("bise") souffle, elle entraîne les polluants à très grande vitesse et le ciel est "nettoyé". Les vents du Sud et de l'Ouest apportent surtout la pluie et la basse pression et leur vitesse est inférieure aux vents du Nord. La situation d'inversion se rencontre en automne et en hiver, et occasionnellement, au printemps. Les couches de stratus situées entre 100 et 300 m au-dessus du sol envahissent l'ensemble du territoire. En hiver, où l'inversion nocturne se prolonge le jour, elle peut durer plusieurs jours consécutifs. C'est à cette occasion qu'on peut voir la pollution atmosphérique atteindre ses niveaux les plus élevés.

3. Variations de la pollution atmosphérique par le SO_2

Vu ce qui précède, on ne s'étonnera pas de constater que la pollution par le SO_2 est caractérisée, en plus de très fortes variations saisonnières, par des fluctuations acycliques, en particulier durant la mauvaise saison. De manière à choisir une valeur qui soit représentative de la période de l'enquête et qui puisse servir de base à la mise au point d'une carte, nous avons appliqué aux relevés provenant des dix postes de mesure, une méthode dite de "désaisonnalisation" (2). Elle permet de mettre en évidence un "trend", une composante saisonnière et une composante aléatoire. A partir de ces calculs, on a obtenu une valeur annuelle pour la période de l'enquête aux différents points d'observation.

4. Indicateurs indirects de la pollution par le SO_2

Résumée dans l'introduction (cf supra), la méthode appliquée sera décrite dans une autre publication (3). A l'occasion d'une régression par pas, 2 paramètres se sont montrés en très forte corrélation avec les valeurs de SO_2 calculées selon la méthode décrite ci-dessus (outre les paramètres météorologiques) : l'un, évident, est la densité de la population, l'autre, inattendu, est le chiffre d'affaires théorique des ramoneurs, qui est proportionnel à la puissance des installations de chauffage. A partir de ces chiffres d'affaires théoriques par hectare, obtenus dans les différentes communes du Canton, nous avons pu dresser une première carte où des zones d'"isopollution" de niveaux croissants recouvrent soit l'agglomération urbaine, soit les zones sub-urbaines, soit les régions rurales, ou semi-rurales.

5. Prévalence de la bronchite chronique et des symptômes respiratoires

1681 hommes et 1201 femmes appartenant aux secteurs de l'industrie, du commerce et de l'administration ont rempli le questionnaire du British Medical Council, traduit en français par la CECA. L'enquête s'est prolongée durant plusieurs mois. Partant de la définition de la bronchite chronique donnée par le Ciba Guest Symposium, nous avons extrait du collectif les bronchitiques sur la base de quatre questions touchant la toux et l'expectoration chronique. Puis nous avons sorti du collectif restant les sujets dits indemnes de symptômes respiratoires. Entre deux se trouvent les sujets présentant des symptômes respiratoires, sans que ceux-ci satisfassent la définition de la bronchite chronique : ils constituent le groupe dit intermédiaire (1). D'après l'analyse que nous avons appliquée au questionnaire (4) les questions sélectionnées par nous se sont révélées particulièrement aptes à discriminer les bronchitiques vrais des non bronchitiques; mais tel n'a pas été le cas pour les questions se rapportant à l'exposition aux intempéries ou à l'exposition aux polluants atmosphériques. Il serait trop tôt pour en conclure que la pollution ou les intempéries n'interviennent pas dans la discrimination entre bronchitiques et non bronchitiques, car on peut aussi mettre en doute la validité des questions qui concernent ces derniers facteurs.

6. Mise en relation des prévalences avec les équivalents de la pollution

Après avoir regroupé les communes en classes selon la densité de la population et le chiffre d'affaires des ramoneurs, on a placé en regard, le nombre de personnes examinées N, le nombre de bronchitiques B, et calculé le taux (en %) de la prévalence $p = \frac{100 \times B}{N}$. On a fait de même pour la population intermédiaire. En vue de déceler une éventuelle relation entre la prévalence p et la densité x d'une part, entre la prévalence p et le chiffre d'affaires des ramoneurs y d'autre part, on a transformé ces prévalences p en probits, puis ajusté à ces probits une régression linéaire dont la variable explicative (indépendante) est soit x, soit y, selon une technique statistique décrite par exemple par Linder (5). A ces ajustements, sont associées deux analyses de variance avec leurs tests de signification (basés sur la répartition du χ^2); elles permettent les observations suivantes :

a. Dans le cas de la densité de la population, le coefficient de la régression ne diffère pas significativement de zéro (seuil : 5 %); la variabilité résiduelle est un peu trop grande, mais ne doit pas être considérée cependant comme significative.

b. Dans le cas du chiffre d'affaire, le coefficient de régression ne diffère pas significativement de zéro (seuil : 5 %) et la variabilité résiduelle n'est pas significative (seuil : 5 %).

c. Quand on combine différents paramètres, représentatifs de la pollution, dont la densité de population et le chiffre d'affaires des ramoneurs, le coefficient de régression devient hautement significatif ($p < 0{,}001$).

D'après ces résultats, on peut prétendre qu'il existe à Genève une relation entre la prévalence de la bronchite chronique et la pollution atmosphérique pour autant qu'on puisse recourir à plusieurs indices de cette pollution. Avoir mis en evidence cette relation paraît d'autant plus intéressant que la prévalence de la bronchite chronique à Genève est basse et que les niveaux de pollution sont faibles.

7. Introduction des données de pollution dans l'analyse typologique

On peut se demander, entre autres choses,si la pollution atmosphérique favorise le développement de la bronchite chronique en tant que maladie spécifique ou si elle agit sur les symptômes respiratoires même banals; si, comme cela a été avancé, son effet est augmenté quand elle se combine à d'autres facteurs exogènes comme la fumée de cigarette. Suffit-il d'être exposé à la pollution pour être atteint ? suffit-il de ne pas être exposé pour échapper à la bronchite chronique ? ou faut-il posséder un terrain prédisposé ? sans permettre de répondre à toutes ces questions, l'analyse typologique offre la possibilité d'en explorer quelques-unes. L'analyse typologique procède à des regroupements successifs d'individus en des types dont ils sont le plus proche suivant une métrique appliquée à des variables-indicateurs. Dans un cas comme le nôtre, chaque réponse au questionnaire est considérée comme une variable, mais aussi les données mesurées comme le débit expiratoire de pointe. Le processus de constitution des types passent par des phases successives. Dans la première, à partir de plusieurs échantillons extraits de façon aléatoire du collectif, le programme construit une typologie de T_{max} types, en analysant toutes les distances entre les individus pris deux par deux. Puis un échantillon plus important est classé selon cette première typologie et une deuxième typologie de T_{max} types est construite ; celle-ci est réduite de T_{max} en T_{min} types. L'utilisateur a alors la possibilité de s'orienter vers le choix automatique du nombre de types le plus approprié à la structure des données ou de l'imposer. La typologie reconnue contient T types avec T compris entre T_{max} et T_{min}.

Le programme d'ordinateur fournit : les distributions de fréquence de toutes les variables considérées ; un profil par type exprimant, pour chaque variable, sa distribution ; une évaluation de la variance expliquée s'arrêtant à la limite que l'utilisateur fixe lui-même selon le degré d'homogénéité qu'il recherche ; le programme attribue de plus, à chaque individu, un numéro de type qui permet de le faire entrer dans une nouvelle catégorie ; c'est ainsi que nous avons pu comparer notre classification a priori, inspirée de la définition du Ciba Guest Symposium, à celle donnée par le programme. A cette analyse s'ajoute une étude topographique des variables ou des types, dans la représentation de l'espace factoriel.

L'analyse typologique a montré que la pollution atmosphérique, telle que nous l'avons définie, était l'un des paramètres qui présentait un fort pouvoir discriminant vis-à-vis de la population étudiée (l'ensemble du collectif sus-mentionné). En d'autres termes, nous avons examiné des sujets aussi bien dans les régions de haute que de basse pollution. Parmi les sujets définis comme bronchitiques chroniques, certains sont exposés à des hauts niveaux de pollution, d'autres pas. Cependant, c'est le groupe qui présente les signes de complication les plus graves qui est soumis aux niveaux les plus élevés. L'association entre forte consommation de tabac et forte pollution ne s'est pas révélée plus active que chacun des deux paramètres pris isolément.

En bref, l'analyse typologique permet de conclure que, dans notre collectif, la teneur en SO_2 ne semble pas avoir joué le rôle d'un facteur déclenchant de la bronchite chronique, mais qu'elle pourrait favoriser l'aggravation des symptômes respiratoires, au moins chez les bronchitiques.

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DISCUSSION

RYLANDER (Suisse)

Est-ce qu'il y a une influence des classes sociales dans votre matériel? On sait bien qu'il y a une influence importante sur la fréquence de la bronchite chronique par les classes sociales.

REY (Suisse)

Toutes les classes sociales ne sont pas représentées dans notre échantillon de la population active mais parmi celles qui s'y trouvent, nous n'avons pas observé de différence dans la prévalence de la bronchite chronique.

BRILLE (France)

Les valeurs de SO_2 prises en compte dans la relation entre bronchite chronique et pollution sont-elles des moyennes annuelles, une moyenne pondérée ou la valeur d'un mois?

REY (Suisse)

Il s'agit de moyennes annuelles calculées sur la base de relevés hebdomadaires exprimés, pour permettre la comparaison entre les différents postes, en mg de SO_2 par 30 jours, déposés sur le papier impregné (méthode Leclerc)[2].

SCHLIPKOETER (République Fédérale d'Allemagne)

Comme vous avez pu déceler, en dépit de valeurs SO_2 très faibles, une augmentation du syndrome de la bronchite à mesure que les concentrations de SO_2 s'élèvent - ce qui est étonnant - il y aurait lieu de vérifier si les différences ne sont pas imputables aux différences de densité démographique. La bronchite peut résulter d'une ou de plusieurs infections qui sont plus fréquentes lorsque la densité démographique est plus forte. A-t-il été tenu compte de la densité démographique dans les calculs de corrélation?

REY (Suisse)

Nous avons étudié la relation entre la prévalence de la bronchite chronique et la densité de la population sans trouver de résultats significatifs comme cela a été indiqué dans le texte.

EPIDEMIOLOGICAL STUDIES OF LUNG DISEASE IN URBAN AND RURAL COMMUNITIES

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ABSTRACT

We have studied over 7000 residents of one urban and two rural communities in the United States, aged 7 years and older, residing in geographically determined areas in which a private census was obtained. The 7000 persons seen comprise about 75% of the defined populations; additional door-to-door surveys provided information about those not seen for validation of the results in terms of the total community populations. A mobile laboratory with an on-line computerized data collection system was used to record answers to a questionnaire on respiratory symptoms, residential and family history, smoking habits and other environmental exposures, and to record maximum expiratory flow-volume curves. Outdoor air quality is being determined over a period of more than one year in each community, using standard methods for particulates, SO_2, NO_2, ozone, sulfate and nitrate. In addition, studies with portable air samplers for respirable particulates, SO_2 and NO_2, were carried out by selected subjects, monitoring their personal environment over 24-hour periods. The results in the urban and in one rural population, both in Connecticut, indicate: (1) there is no significant excess of respiratory symptoms among lifetime urban dwellers, living in an area where particulate pollution exceeds the primary AQS in 39% of 102 samples; (2) sensitive lung function tests do not show differences attributable to an increased prevalence of

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airway obstruction among urban adults when compared to rural dwellers; (3) indoor particulate pollution may add considerably to the total respirable particulate load; (4) occupational or domestic, toxic and allergenic inhalants, including tobacco smoke, are important etiological factors in many persons with airway obstruction; outdoor air pollution may only play a minor role.

1. Introduction

Epidemiological studies on chronic bronchitis and other lung diseases in communities have generally used population samples. Such samples often contain too small numbers of persons in defined, homogeneous subgroups to allow study of more than a few variables. Computerized methods now allow us to broaden the scope of such surveys to total community populations, to a wider range of questions concerning lung disease, and to more sophisticated lung function tests. We have developed a mobile computerized laboratory, in which standardized, computer-prompted interviews on respiratory symptoms and environmental exposures (for text of questionnaire, see Bouhuys [1]) as well as lung function testing are conducted simultaneously on 2 terminals connected to a PDP-8E computer, using a time-sharing system. With this facility, we have recorded symptoms and lung function data in about 2500 residents of a rural town (Lebanon) and 1500 residents of an urban area (Ansonia), both in Connecticut, U.S.A. Similar data have recently been obtained in about 4000 residents of a rural town (Winnsboro) in South Carolina (Feb.-May 1974). In each community, a total population census as well as door-to-door surveys in selected areas provide information on persons not seen in the mobile laboratory, thus enabling us to establish the validity of our findings in terms of the total population. An air sampling network with 3-5 stations is used to characterize outdoor air quality, using standard methods recommended by the Environmental Protection Agency. 24-hour samples are analyzed for suspended particulates, sulfate and nitrate, as well as for SO_2, NO_2 and ozone. In the two Connecticut towns, samples were obtained at least weekly; in Winnsboro, S.C., daily samples are obtained during four 3-week periods in the fall, winter, spring and summer. This schedule of sampling defines the outdoor pollution load over a year.

This paper describes initial results of the surveys in Lebanon and Ansonia, Conn., and of air quality measurements in these communities. In addition, preliminary data on 24-hour personal environmental sampling to compare individual pollutant exposure and outdoor air quality are included.

2. Lung function tests. We have selected the maximum expiratory flow-volume (MEFV) curve as a simple, objective test of ventilatory function which provides: (a) standard spirometric values, (b) measurements of flow rates at low lung volumes, which are sensitive indices of airway obstruction, and (c) visual recognition of obstructive and restrictive function loss (see review in Bouhuys [1]).

The mobile laboratory is equipped for simultaneous recording of MEFV curves on two terminals, using Fleisch pneumotachographs to measure expiratory flow rates. Flow signals are digitized and stored by the computer, which also integrates flow to volume, presents each MEFV curve on the graphic terminal, and calculates forced vital capacity (FVC), forced expiratory volumes (FEV_1 and FEV_3) and peak expiratory flow rate (PEF) from the 2 blows with the highest $FEV_{1.0}$ in a series of 5 blows. Average maximum expiratory flow rates at 50% and at 25% of the FVC (i.e., total lung capacity minus 50 or 75% of the FVC) were measured from the same two blows. In contrast to PEF, these flow rates (MEF50% and MEF25%) are largely independent of muscular effort; while PEF reflects tracheal size and effort, MEF50% and MEF25% depend primarily on the caliber of small airways. Quality-control features of the software include rejection of too short blows, and calibration and zero drift correction subroutines. Precision flowmeters, as well as a newly designed motor-driven 5-liter syringe which delivers air at varying rates simulating an MEFV curve, are used to calibrate the volume and flow measurements and to check the computation subroutines. For a more detailed description of the system, see Bouhuys [1].

Output format. After the questionnaire and MEFV curves have been recorded, data output is obtained (a) in a condensed hard copy format for immediate review and checking, (b) on punched paper tape, for editing and transfer to magnetic tape on a home-based PDP-11 computer which classifies the results in tabular form and performs statistical analyses.

3. Personal environment sampling. To obtain air quality data that may more adequately reflect an individual's changing environment, we have developed a portable light-weight and low-noise personal environment sampler (PES) that allows individuals to monitor their environment during 24 hours (Hosein et al. [2]). The design is a modification of an instrument developed by Burgess et al. [3]. The unit is housed in a suitcase, which contains a blower drawing in air at 250 liters/min. From the mainstream, individual sampling pumps draw air through collection vessels for SO_2 and NO_2, and through a membrane filter for respirable particulates (approx. <7μm), using a cyclone.

4. Results. *Symptoms and lung function*. Analysis of data obtained in door-to-door surveys in selected areas of each town has shown similar symptom prevalences among those seen in the mobile laboratory and those not seen, if men and women of similar age are compared. These results suggest that our results can be extrapolated to the total community population.

There were no significant differences between the prevalences of persistent cough and phlegm, frequent wheezing and dyspnea on exertion among rural and urban males (age 7-80 yrs); their smoking habits were similar. The prevalence of persistent cough and phlegm was similar among rural and urban nonsmoking men and women (25-64 yrs), except that older urban women (45-64 yrs) more frequently reported persistent phlegm ($\chi^2 = 5.26$, $p < 0.05$). The prevalence of these symptoms was higher among current smokers (men and women, 25-64 yrs), without significant differences between rural and urban dwellers.

The lung function data showed similar differences. For instance, $FEV_{1.0}$ and MEF50% were lower in smokers than in nonsmokers but there were no significant differences between nonsmoking or smoking rural and urban men or women, 25-64 yrs. In this analysis, stature was taken into account by including only data from 150-169 cm tall women and 160-179 cm tall men. The interpretation of lung function in younger persons (7-24 yrs) requires a more complex analysis of the simultaneous effects of growth, smoking and urban or rural residence; this has not yet been completed. Thus, we have found no evidence of increased airway obstruction among urban adult men and women when compared to rural residents, using a sensitive lung function test (MEF50%) which, e.g., detects significant airway obstruction in teenaged smokers (Seely et al. [4]).

Outdoor environment sampling. In Lebanon (rural), weekly SO_2 and ozone levels were always less than about 50% of the primary air quality standard (AQS), while total particulates and NO_2 exceeded the AQS in, respectively, 2 and 10% of all samples taken over a 42-week period. In Ansonia (urban), ozone was always less than 50% of the AQs, while particulates, NO_2 and SO_2 exceeded the AQS in, respectively, 39, 35 and 1% of all samples taken over a 42-week period. Ansonia, situated in the heavily industrialized Naugatuck valley, is generally considered a polluted town, and our measurements confirm that particulate and NO_2 levels frequently exceed those in rural Connecticut.

Personal environment sampling. The highest outdoor total particulate level measured in Ansonia was 144 $\mu g/m^3$. The personal environment samplers (PES) frequently indicated higher respirable particulate levels (up to 303 $\mu g/m^3$) when they were carried about by selected residents for 24 hours, covering their normal daily indoor and outdoor activities, within 2 miles from the outdoor samplers. In general, SO_2 and NO_2 levels with the PES were less than those in outdoor air. Thus, indoor environments may add considerably to the total respirable particulate load. In two instances, homes with very high particulate levels (up to 450 $\mu g/m^3$) were identified through the clinical data of their inhabitants, which suggested airway obstruction unexplained by smoking.

5. Conclusions. This is an interim report on a still ongoing study. In the data analyzed thus far we have not found evidence to warrant the conclusion that urban outdoor air pollution in a typical industrialized U.S. town is a significant causal factor in the pathogenesis of lung disease, as evidenced by respiratory symptoms and obstructive lung function loss. Maximal expiratory flow rates at low lung volumes, which are highly sensitive indicators of airway obstruction in smokers, textile workers, hairspray users and others exposed to airway constrictor inhalants, are if anything higher rather than lower among groups of urban dwellers than among their rural counterparts, smokers or nonsmokers. There are indications that indoor air pollution may be a more significant factor in the pathogenesis of airway obstruction than outdoor air pollution. A complete analysis of smoking habits, occupational and domestic exposures, as well as of histories of allergy, in relation to respiratory symptoms and objective, sensitive and quality-controlled lung function test data, is required before a causal role of outdoor air pollution in the pathogenesis of obstructive airway disease can be established.

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DISCUSSION

GOLDSMITH (U.S.A.)

The manuscript reports two interesting findings which suggest that community pollution and indoor pollution may produce impairment of lung function. The first is the low level of $FEV_{1.0}$ in urban non-smoking women. The second in the "clinical data" and impairment of lung function in persons with high indoor particulate matter. Could you give any further information on these points?

BOUHUYS (U.S.A.)

1. The $FEV_{1.0}$ values of urban and rural non-smoking women did not differ significantly. There is no evidence for impairment of lung function by community air pollution in our study.

2. Details on our studies on indoor air pollution could not be included in the paper for lack of space. They will be published elsewhere.

USES OF MORTALITY AS A MEASURE OF THE HEALTH EFFECTS OF AIR POLLUTION

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ABSTRACT

The data obtained in a 13 year follow-up of mortality in two communities subjected to widely different air pollution levels are used to compare the results of different methodological techniques in the study of mortality differences. Factors are derived from the data to estimate the error which would result from neglecting to consider the effects of smoking and residence history.

There were greater differences in mortality rate within each community due to smoking than between the two communities. The higher mortality rates of smokers makes comparisons between populations with similar heavy smoking habits a more powerful test of mortality differences due to air pollution than comparisons of populations with mixed smoking habits. Increased length of residence in the highly polluted community was associated with increased age-adjusted mortality. The reverse was true in the control community, suggesting a process of self-selection in inmigration.

The findings demonstrate that differences in the prevalence of smoking or in length of exposure to air pollution could affect the validity of studies not taking these factors into account. The degree of the resulting error is estimated.

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1. Introduction

The Preamble to the Constitution of the World Health Organization defines health as "...a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." Although this definition is widely accepted in principle, most assessments of health effects are much more limited in scope due to the inability to develop suitable measurements to determine the presence, absence or degree of "health." Mortality statistics are often considered too crude and non-specific for the analysis of health effects, yet this non-specificity which may complicate analytical techniques supports their use as a measure of this broad concept of health. As Taeuber suggests, mortality rates reflect cumulative stresses, deprivations of place, milieu, and life-style, along with insufficiencies of medical care and health guidance[1].

2. Importance of Various Parameters of Mortality

Tabulations of deaths by cause often present difficulties of interpretation due to unreliable and varying diagnosis and artificialities introduced in the coding process. Information on cause of death may help to estimate the impact in terms of individual suffering and societal costs in the care of terminal illness. It can also be useful in establishing cause-effect relationships between environmental stress and increased mortality. The fact that one population may have more heart disease or more cancer mortality than another is certainly useful in analyzing the impact of environmental stress. But the major measurement of impact must be: does one population suffer a higher expectancy of mortality within the age groups that represent substancial losses to society. As important as cause of death is in the epidemiology of disease, it is of only supplimentary value in the assessment of health effects. Age at death is a much more important factor in evaluating the impact on society than cause. The total number of deaths within suitably narrow age groupings are also available for most countries to a high degree of accuracy.

3. Design of Mortality Study

The design of epidemiological studies of the effects of air pollution on mortality have been generally temporal or inter-area comparisons. The availability of data from a study of the effects of air pollution on respiratory function done 13 years before by others [2] provided us with the opportunity to conduct retrospectively a long term follow-up of mortality in two populations exposed to widely different air pollution levels. Subgroups of the two populations were established based on age, sex, smoking history, and residence (exposure) history at the beginning of the observation period. **Unfortunately, data on occupation and socioeconomic status were not available.**

4. Analysis

Alternative approaches were taken to the analysis of the data. The general approach was to compare the observed number of deaths with the expected in each population subgroup by means of the standard mortality ratio (SMR). The expected deaths were determined by applying rates from the 1965 U.S. white population. Two methods were employed. The first determined the expected deaths over the study period by determining the effective population exposed to the risk of death at each age level and multiplying by the standard population mortality rate for that age. Mancuso and Coulter explain this method in detail[3]. By reducing exposure to terms of person-years, this method equates a small population observed over several years to a larger population over one year, allowing easy comparisons between populations with different observation periods. It also allows easy inclusion of information on individuals who enter or leave the population at any time during the study. Since the mortality pattern in the sample itself helps to shape the exposure, it has some influence on the denominator as well as the numerator of the SMR.

Table 1 presents the data developed for this analysis.

SEWARD-NEW FLORENCE

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Summary of Data by Smoking and Residence History

	Length of Residence					
	Over 20 Years			Less Than 20 Years		
SEWARD	Person-Years	Obs	SMR	Person-Years	Obs	SMR
Males						
Never smoked	375.5	9	63	122.5	0	0
Intermediate	669	24	106	395	9	99
Heavy	605	18	190	512.5	4	65
Females						
Never smoked	1258	20	76	771.5	3	47
All smokers	413.5	1	36	431	4	215
NEW FLORENCE						
Males						
Never smoked	228.5	4	49	227.5	2	62
Intermediate	423.5	17	102	286.5	5	101
Heavy	400	10	154	375.5	7	186
Females						
Never smoked	1098	14	50	656	8	60
All smokers	429	0	0	182	0	0

Total person-years observed: 9860.

TABLE 1.

The second approach was to calculate the *a priori* expection of mortality from the standard population life table, based on age and sex at the beginning of the observation period. Mortality patterns during the study then are independent of the expected number of deaths. This approach also allows analysis of generational effects.

In addition to the use of SMRs, survival curves were used as described by Cutler and Edera 4 . These curves show the fraction of the population surviving over time. Numerical comparison of the slope of these curves (linearized on a semi-log plot) for different subgroups of the population show differences in the rate of mortality.

5. Findings and Discussion

The findings of the study indicate a significant 2-fold difference in mortality rate between heavy cigarette smokers and non-smokers in the adult male population. Differences between smokers and non-smokers within each community were greater than the differences between communities. An increased mortality rate among long time residents of the highly polluted area was suggested. Increased length of residence in the high pollution community was associated with increased age-adjusted mortality. The reverse was true in the control community, perhaps suggesting a self-selection process in in-migration.

The primary parameters influencing mortality rates are age and sex. These must be taken into account when assessing the differences in mortality due to other factors. Quantitatively, the difference in mortality rate for long time adult residents apparently due to air pollution between the two communities was 3.8/1000, or 0.09/1000 per ug/m^3 total suspended particulate air pollution difference. If it is assumed that there is no effect in the more recently arrived population (actually, the data indicate a reversal of the effect in this group) than each unit increase in the percentage of the population with more than 20 years residence leads to an increase in the overall adult mortality rate of 0.04/1000 or 0.001/1000 for each ug/m^3 difference in suspended particulates. Under this hypothesis, 10 percentage points reduction in the percentage of the population with over 20 years residence could reduce the mortality rate observed in the general population by 20 percent.

Each unit increase in the percentage of heavy smokers in a community resulted in a change in the general adult mortality rate of 0.12/1000. Thus, a difference of 10 percentage units in the fraction of heavy smokers in the two communities could result in a difference in mortality of 1.2/1000, or about one-third of the difference due to air pollution.

6. Conclusion

Mortality data provide a useful index of the total health of a population. They are thus a useful tool in the assessment of the health impacts of environmental stress on a population. The fact that the stress may work primarily through specific subgroups of the population and the action of other concurrent forms of stress can confound the results. Thus, differences in the relative size of the group primarily affected by air pollution in the population can result in spurious differences in the effects on the total population mortality rate. Differences in the fraction of the population that are heavy cigarette smokers, if not accounted for, may mask or imitate air pollution effects.

By utilizing factors developed here, cohort studies can be limited to groups for which high mortality rates are expected. Significant differences due to air pollution, where they exist, may then be obtained by the use of smaller, but more carefully selected, samples. Of more general interest, we believe that these factors can provide estimates of the error inherent in studies which do not include data on smoking or residence history.

7. Acknowledgements

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DISCUSSION

SULAIMAN (Nigeria)

Most participants give details on the impact of smoking and general air pollution. How can we remove the effect of home environment factors from these atmospheric air pollution studies?

MORRIS (U.S.A.)

This is a very important point and one on which considerably more study is needed. To take home exposures into account we must divide the study population into groups based on level of indoor pollutants in the home. This could be done by questioning the individual subjects as to the type of equipment and fuel used for heating and cooking, presence of smokers in the home, etc. Then a sampling of homes in each subgroup must be monitored to determine variations in exposure. I was especially pleased to see Professor Bouhuys present some data of this nature in his paper.

WASSERMAN (Israel)

Some surveys are needed for (characterizing) identifying particular sources of air contamination which may cause a high incidence in respiratory illness. With reference to Solaiman's remark, it should be observed that in some African houses, the babies are carried on the vack of their mothers until the age of two, while they are cooking in the home with green wood. These children are inhaling high amounts of smoke, which - as observed in cases of necropsy - impregnate their lungs. Thus, in a survey, the rate of illness will be reported to cooking facilities in the respective homes.

L'INFLUENCE DES POLLUTIONS INDUSTRIELLES DE L'AIR SUR L'ORGANISME HUMAIN

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RESUME

On a examiné la population habitant à proximité des fonderies de cuivre et à titre de comparaison - les groupes de contrôle de la population n'étant pas exposées aux pollutions industrielles. Chez les sujets exposés la concentration de plomb dans le sang était remarquablement élevée. On a constaté une diminution des activités de ALAD, LDH, Ald., G-6-PD dans les érythrocytes ainsi que le taux des haptoglobines significativement abaissé et celui d'alpha-1-antitripsyne - élevé. Les examens hématologiques ont démontré un taux abaissé de Hb et le nombre diminué d'érythrocytes ainsi que le pourcentage plus élevé des réticulocytes dans les groupes des personnes exposées au danger de pollution industrielle.

Aujourd'hui, avec le brusque développement de l'industrialisation, le problème de la santé humaine menacée par des facteurs physiques, chimiques et biologiques devient de plus en plus actuel et imminent. L'objectif de notre travail est la détermination de l'influence des oxydes de plomb, des oxydes de cuivre et d'autres facteurs toxiques de l'atmosphère sur l'état de la santé et sur les processus métaboliques de la population habitant à proximité des fonderies de cuivre.

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ABSTRACT

Population groups living in the vicinity of copper foundries were examined and compared with control groups composed of subjects not exposed to industrial pollution. In the subjects exposed to pollution the lead concentration in the blood was remarkably high. We recorded a decrease in ALAD, LDH, Ald. and G-6-PD activity in the erythrocytes, a significant drop in the number of haptoglobins and a high alpha-1-antitrypsin count. The haematological tests revealed a decrease in Hb and erythrocytes and a higher percentage of reticulocytes in the groups of people liable to exposure to industrial pollution.

In these days of rapid industrialization the problem posed by the physical, chemical and biological threat to human health is becoming more and more immediate. The aim of our work is to determine the effect of lead oxides, copper oxides and other toxic substances in the atmosphere on the health and metabolic processes of population groups living in the vicinity of copper foundries.

MATÉRIEL ET MÉTHODES

Les populations suivantes constituent le matériel de nos recherches:

- Groupe I : habitants aux environs de la fonderie de cuivre en marche depuis 10 ans environ (122 sujets);
- Groupe II : habitants aux environs de la fonderie de cuivre en marche depuis 2 ans environ (121 sujets);
- Groupe III : habitants d'une grande ville, des hommes, exposés professionellement aux gaz d'échappement (92 sujets);
- Groupe IV : groupe de contrôle englobant les habitants de la campagne, pratiquement non exposés aux pollutions industrielles (134 personnes).

Au total, on a examiné 469 cas.

Les personnes en question ont passé des examens médicaux de routine, en plus, examen radiologique du thorax, examen de l'urine, ECG, examen otolaryngologique. On a procédé à une minutieuse enquête concernant les conditions de leur vie quotidienne, leurs indispositions subjectives, leur travail professionel, leur contact avec des produits chimiques, leurs maladies antérieures et défauts congénitaux, ainsi que leur charge familiale.

On a déterminé le taux d'hémoglobine (Hb), la valeur de l'hématocrite, le nombre d'érythrocytes et de leucocytes, la formule sanguine, le nombre de réticulocytes et le nombre de plaquettes sel. Rees-Ecker (1). La concentration du plomb total du sang a été effectuée d'après Dutkiewicz (2), celle du cuivre dans le sérum sel.Chiesura (3), l'activité (l'act.) de cholinestérase (CHE) sel. la méthode de Hestrin en modification de Huerg-Yesinic-Popper (4), l'act. de déhydratase de l'acide delta-aminolévulinique (ALAD) sel. Bonsignore (4), l'act. de gamma-glutamyl-transpeptidase (GGTP) sel. Orlowski (5), l'act. des aminotransférases (ASPAT, ALAT) dans le sérum d'après Reitman-Frank (6), l'haptoglobine sel. Jayle (7), la céruloplasmine sel. Ravin (8), l'alpha-1-inhibiteur de tripsy syne sel. Homolka (9). L'act. de la phosphatase alcaline (Ph.alc.) et acide (Ph.ac.) a été déterminée sel. Sigma Tech,. Bull. (10), l'act. de l'aldolase (Ald.) dans les érythrocytes sel. Bruns (11), l'act. de la déhydrogénase glucoso-6-phosphate (G-6-PD) suivant Richterich (12), l'act. de la déhydrogénase lactique (LDH) par la méthode de Zimmermann et Weinstein (13), l'électro-

Tableau II. Comportement des indices biochimiques /moyennes arythmetiques/.

	Groupe I	Groupe II	Groupe III	Groupe IV
Concentration de Pb dans le sang μg/100 cm^3	182	76	114	42
Concentration de Cu dans le plasma, μg/100 cm^3	274	231	-	138
Serum:				
Haptoglobines mg/100 cm^3	106,1	122,9	116,2	144,4
Ceruloplasmine mg/100 cm^3	44,4	37,4	40,7	43,2
Pseudochol-est. I.U.	2,0	1,8	1,2	2,3
ASPAT " "	15,3	17,6	41,5	15,3
ALAT " "	12,3	9,7	34,0	9,4
Alk.phosph. " "	2,0	2,1	0,91	1,9
Acide phosph. " "	0,9	0,6	-	0,8
α-1 inhibiteur de tripsyne	1344,0	1559,1	1251,6	1302,4
Erythrocytes: /I.U./g Hb/				
LDH	23,6	20,0	20,0	27,3
Aldolase	0,99	0,75	1,2	1,14
G-6-P Deh.	4,0	3,0	4,0	4,2
Acethylcholest.	6,0	6,6	4,5	7,2
ALAD	3,3	8,0	6,6	16,6
ASPAT	0,83	0,93	1,75	0,75

phorèse des iso-enzymes (LDH) sur gel polyacrilamidique sel. Davis (14). On a évalué les moyennes arithmétiques et les déviations-standard; les différences entre les moyennes ont été déterminées suivant le test "t" Student afin d'y trouver la différence valable.

LES RESULTATS DE RECHERCHES ET DISCUSSIONS

Dans les groupes I et II on notait souvent des plaintes contre la fumée, contre la mauvaise odeur dans l'air, et dans les groupes de femmes, contre les nausées et les douleurs dans la région de l'hypochondre. Les groupes I et II présentaient en général un état de santé moins bon. On a trouvé dans ces groupes des changements de la muqueuse de la cavité buccale et du nez (25 - 30 %) de type allergique avec endommagement de l'odorat, ou bien des changements dans l'avant-bouche et des gencives ressemblant à la leucoplasie.

Les résultats de recherche hématologique ont été groupés dans le tableau I.

Parmi les femmes du groupe I et du groupe II, le taux de Hb et le nombre d'érythrocytes étaient en effet plus bas que dans le groupe de contrôle. Ce qui est frappant chez les hommes du groupe I, c'est le taux abaissé de Hb; le taux de Hb le plus élevé et le plus grand nombre d'érythrocytes démontre le groupe urbain. Le nombre de plaquettes chez les femmes était le plus bas dans le groupe II où le temps de menace était plus court. Le pourcentage des réticulocytes dans les groupes I, II et III était significativement plus élevé que dans le groupe de contrôle.

Dans le tableau II on a montré le comportement de facteurs biochimiques estimés par nous comme les plus sensibles dans l'évaluation du danger. La concentration de plomb dans le sang était la plus élevée dans le groupe I et différait de manière significative des valeurs moyennes d'autres groupes. La concentration de cuivre dans le sérum du groupe I et du groupe II était deux fois plus élevée que dans les groupes de contrôle. L'analyse des résultats de recherche sur les protéines et sur les enzymes démontre un abaissement du taux des haptoglobines statistiquement significatif, ainsi que la diminution de l'activité de CHE sérique et l'augmentation du taux d'alpha-1-inhibiteur de tripsyne dans les groupes I et II.

Tableau I. Résultats des examens hématologiques /moyennes arithmetiques et deviations standard/ ($\bar{x}$) (δ)

Groupe, d'examinés sexe /nombre de cas /		Facteur examiné									
		Taux d'hémoglobine en g%		Nombre en mm^3 / x 10^3 /						Réticulocytes en ‰	
				Erythrocytes		Leucocytes		Plaquettes			
		$\bar{x}$	δ	$\bar{x}$	δ	$\bar{x}$	δ	$\bar{x}$	δ	$\bar{x}$	δ
gr.I	fém. /36/	11,6	1,1	3 952,0	0,3	6,3	2,3	127,8	33,8	10,5	10,7
	masc./78/	12,8	1,3	4 280,0	0,4	7,4	2,2	146,6	40,6	10,5	6,3
gr.II.	fém./63/	11,2	1,1	3 793,0	0,8	6,9	1,5	105,4	52,6	11,4	5,4
	masc./54/	14,5	0,9	4 009,0	1,7	6,0	1,6	130,1	43,3	9,6	5,3
gr.III	masc./91/	15,6	1,3	4 703,0	0,5	5,6	1,3	150,1	50,1	8,0	4,3
gr.IV	fém./41/	12,2	1,4	4 181,0	0,8	7,0	2,7	127,6	40,1	6,4	3,5
	masc./91/	13,7	1,2	4 532,0	0,6	7,0	2,3	123,6	46,0	6,3	3,3

On a constaté des changements nets dans les enzymes érythrocytaires chez les sujets exposés, à savoir: l'act. ALAD était remarquablement diminuée, à part cela, dans le groupe exposé à l'action des facteurs nocifs pendant un court délai de temps l'inhibition correspondait à 50 % et, dans le groupe exposé au danger plus longtemps, elle correspondait à 75 %. La diminution de l'act. de ACHE était la plus marquée dans le groupe urbain exposé au danger de vapeurs de plomb tétraéthyle. Les activités de G-6-PD, d'Ald., et de LDH étaient significativement plus faibles dans les groupes exposés. L'étude de l'isozymie LDH d'érythrocytes a démontré des déplacements nets orientés vers LDH$_4$ et LDH$_5$.

L'évaluation de micro-intoxication des populations humaines est un problème complexe par rapport au nombre élevé des facteurs agissant ainsi qu'au manque des manifestations cliniques nettes. Néanmoins, les systèmes spécifiques d'enzymes sensibles au plomb et au cuivre, que nous avons choisi pour nos recherches, semblent le mieux refléter le degré de danger auquel est exposé la population, d'autant plus qu'ils sont en corrélation avec les changements hématologiques. Les troubles enzymatiques d'érythrocytes peuvent être précurseurs ou bien accompagner les changements morphologiques des érythrocytes, elles peuvent également témoigner des troubles dans le métabolisme cellulaire d'autres tissus de l'organisme.

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DISCUSSION

HERNBERG (Finland)

Les teneurs en plomb que vous avez trouvées dans tous les groupes sont si élevées qu'il ne peut s'agir que d'une très grave erreur méthodologique. Des dizaines d'études portant sur les populations et utilisant des méthodes contrôlées ont fait ressortir des teneurs moyennes en PbB d'environ 15-30 mg/100ml, qui, même au voisinage des sources de pollution, dépassent rarement 40 mg/100ml. L'erreur méthodologique, qui doit être, pour le moins, d'un ordre de grandeur, ôte, toute valeur à vos conclusions.

KOTLAREK-HAUS (Pologne)

La méthode que nous utilisons est une méthode de Dithizone (diphénylthiocarbazone) effectuée dans le sang total. Les valeurs normales dans cette méthode sont comme celles pour notre groupe de controle; elles sont un peu plus élevées en comparaison à la méthode AAS. Notre méthode est néanmoins conforme aux valeurs obtenues par d'autres autres utilisant cette méthode, dont les valeurs s'élèvent à 60-70 mcg/100 ml du sang. (1) Cradwohls, Clinical Lab. Methods and Diagn. Ed. by Fraenkels., Reitman S., C.W. Mosby Comp. 1970; (2) Kehoc R., Arch. Environ. Health 1964, 8, 232; (3) Christian G.D.: Analyt. Chem. 1969, 41, 274.

Dans notre groupe de contrôle la teneur en plomb dans le sérum s'élevait à 10 mcg/100 ml.

ZIELHUIS (Pays-Bas)

Le tableau I fait ressortir des différences, par exemple dans les niveaux d'hemoglobine et dans les nombre d'érythrocytes. La comparaison des groupes du point de vue des effets de leur exposition à l'environnement ne peut se faire que si les groupes sont par ailleurs comparables en ce qui concerne les conditions de nutrition, les conditions socio-économiques, le milieu ethnique, les pressions barométriques, l'âge. Cette étude satisfait-elle à ces conditions?

KOTLAREK-HAUS (Pologne)

Les groupes examinés étaient comparables par rapport aux moyennes d'âge, et à la distribution dans les classes particulières d'âge; leurs conditions socio-économiques et géographiques n'étaient pas évidemment les mêmes, mais pareilles. Toutes ces données ont été évaluées par les statisticiens quand à leur validité.

BERLIN (C.E.C.)

1. Dans votre communication il est indiqué que les taux de plombémie pour les personnes du groupe III (habitants des villes professionnellement exposés) sont beaucoup plus élevés que pour ceux du groupe II (habitants aux environs d'une fonderie de cuivre en marche depuis 2 ans). Quelle était la profession des personnes du groupe III?

2. Vu ces taux de plombémie très élevés quelles sont les actions entreprises pour les réduire? A partir de quel niveau de plombémie une action prophylatique est-elle mise en route?

KOTLAREK-HAUS (Pologne)

1. Les personnes du groupe III étaient des employés de stations service et des membres de la police routière travaillant dans les endroits les plus pollués de la ville.

2. Ils ont été soumis à la surveillance spéciale, médicale et prophylactique.

EPIDEMIOLOGIC STUDIES OF DDT AND DIELDRIN RESIDUES AND THEIR RELATIONSHIP TO HUMAN CARCINOGENESIS

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ABSTRACT

In contrast to dieldrin residues serologic and adipose surveys of the human DDT residue in the population of South Florida exhibited significant demographic characteristics in both tissues. Total DDT residues were higher in blacks than whites, were age and social class dependent; higher residues being found in the less affluent in both races. Similarly, geographic distribution differences have been identified. These demographic and geographic distribution frequencies emphasize the significant contribution of non-dietary sources of DDT in incidental DDT pollution and the identification of clustering within homes and environmental studies in a Bahamian island population implicated household dust as a major contributant to this type of pollution in tropical areas. When adipose surveys were stratified for these differences no significant differences in Total DDT adipose residue were observed in autopsy and biopsy comparisons from 122 cancer cases when compared with race, sex and social economically matched controls. Similarly, in separate comparisons of these residues in 24 lung cancers, 14 gastrointestinal cancers, 29 breast cancers, and 15 generalized metastatic carcinomas, no significant differences were observed. The complexities of case-control residue studies are described.

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1. Introduction

In contrast to the frequency distribution of human dieldrin residues several studies of the frequency distribution of human DDT studies in both fat and blood indicated that this pesticide residue is not homogeneously distributed throughout the general population. Earlier reports emphasize the homogeneity of this residue a frequency which reemphasized the concept that incidental human exposure was primarily dietary. Frequency distribution studies of the DDT residue in the general population of South Florida, which enjoys a semi-tropical climate, exhibited significant demographic differences in both adipose and serological surveys. Total DDT residues were higher in blacks then whites and were strongly age dependent in the first two decades of life but less so in subsequent age groups, exhibited family clustering and was strongly correlated with social class in the greater Miami area when this parameter was measured by the Hollingshead Index, the census tract population density or the census tract median income (Davies et al [1]). These socio-economic differences which were noted from a serological survey of 581 adults in the general population suggested that higher DDT residues occurred in the less affluent members of the general population in both races. Geographic frequency distribution of DDT but not dieldrin has also been recognized in sizeable adipose DDT residues in the United States. In a prevalence survey of DDT and dieldrin adipose residues from 4,469 members of the general population from 22 States in the United States in 1968, the mean Total DDT residue was 4.85 ppm in whites residing in the cooler states and 9.21 ppm from whites in the warmer states; in contrast the mean dieldrin residue was 0.13 ppm in both areas of the continent. From adipose reisudes of 804 blacks, the mean Total DDT was 7.68 ppm in the cooler states and 14.37 ppm in the warmer states whereas the mean dieldrin level was 0.14 ppm and 0.13 ppm in the cooler and warmer states respectively. These demographic and geographic distribution frequency differences for the human DDT residue emphasize the significant contribution of non-dietary sources of DDT in incidental human DDT pollution, and the socio-economic differences, domestic clustering and environmental studies in a Bahamian island which implicated the important role of household dust, reinforce the importance of giving due consideration to the micro-epidemiologic variables of the home environment in future epidemiologic studies which may compare the organochlorine pesticide residues in health and disease. Having these stratification requirements in man, adipose organochlorine levels in 122 cancer cases were compared with residues from 122 controls obtained from persons coming to surgery or dying from non-malignant cases, matched for age, race, sex and social class. This paper reviews the results of this study and also describes the effect of social economic class in this adipose organochlorine residue study.

2. Materials and Methods

Serological and biopsy fats were obtained from 122 cancer cases coming to surgery or autopsy on account of the following malignancies: primary lung cancer, gastrointestinal cancer, breast cancer and generalized metastatic carcinomatosis. Similar specimens were obtained from 122 matched controls coming to surgery or autopsy for non-malignant conditions. Organochlorine levels were obtained by gas liquid chromatography studies on a wet weight basis using the modified Mills procedure [2]. On the few occasions when the amount of fat was insufficient for this method a micro-Mills analyses was performed and the results calculated on a lipid extractable basis. No statistical difference in residues by either method had been previously detected. In addition to matching on the basis of race, sex and social class, and with few exceptions by age, within a 10 year period, information on significant weight loss was categorized into to three categories. Category I - moderate to severe (greater than 12 pounds or a pathologist's report of emaciation), Category II - less than 12 pounds or no weight loss, and Category III - where weight loss was not recorded.

3. Results

In the comparison of adipose residues in 122 matched cancer patients and controls the mean Total DDT was 7.8 ppm in the controls and 8.0 in the cancer patients, a difference which was not significant at .05 level. The mean dieldrin residue was 0.3 ppm in both cancer cases and controls but as may be expected the degree of weight loss in the cancer cases was significantly greater than that observed in the controls.

Table I compares the adipose residues in the four individual types of cancers with matched controls. The data was further sub-divided as to whether metastatic spread of cancer had occurred or not. Again, there were no significant differences with regard to adipose concentrations of Total DDT and dieldrin residues. Significant weight loss was also observed in the individual cancer groups when compared to the con-

TABLE I - Comparisons of adipose pesticide residues (ppm) in groups of matche cancer patients and controls. Dade County, Florida, 1972.

GROUP	n	$\bar{x}$ Total DDT	$\bar{x}$ Dieldrin	Weight Loss
PRIMARY LUNG CANCER				
Controls	24	8.9	0.3	
Cancer	24	8.5	0.2	
"p" Value		NSD*	NSD	NSD
PRIMARY LUNG CANCER WITH METASTASIS				
Controls	22	7.9	0.2	
Cancer	22	9.6	0.4	
"p" Value		NSD	NSD	<.001
PRIMARY GASTRO-INTESTINAL CANCER				
Controls	46	8.4	0.3	
Cancer	46	8.9	0.3	
"p" Value		NSD	NSD	<.001
PRIMARY GASTRO-INTESTINAL CANCER WITH METASTASIS				
Controls	4	7.0	0.3	
Cancer	4	10.5	0.2	
"p" Value		NSD	NSD	<.05
PRIMARY BREAST CANCER				
Controls	29	7.1	0.2	
Cancer	29	5.4	0.2	
"p" Value		NSD	NSD	<01
PRIMARY BREAST CANCER WITH METASTASIS				
Controls	10	5.2	0.1	
Cancer	10	4.6	0.2	
"p" Value		NSD	NSD	NSD
METASTATIC CANCER				
Controls	4	13.7	0.6	
Cancer	4	14.8	0.4	
"p" Value		NSD	NSD	NSD

*No significant differences at .05 level trols yet this weight loss differences had no effect on the average pesticide residue concentrations.

The pesticide residue data in both cancer and controls was also studied with regard to exploring socio-economic class differences measured by the Hollingshead Index [3]. As had been previously noted in our serological survey Total DDT residues were here again significantly greater in the less affluent; dieldrin levels were greater in Social Class 4 and 5 than in Social Class 1 and 2 in the controls (Table II).

4. Discussion

The fact that pesticide residue concentrations were not significantly different in cancer cases when compared with matched controls can be considered to be reassuring or to say the least, and in this small study the data suggests that the incidental environmental exposure to DDT and dieldrin was the same in these selected cancer cases as the matched controls. Earlier reports of similar types of studies have reported conflicting results, with some studies finding significantly levels of

TABLE II - Total DDT and dieldrin residues (ppm) in adipose tissue by social class groups. White cancer patients and controls, Dade County, Florida, 1972.

SOCIAL CLASSES		Total DDT (ppm)			Dieldrin (ppm)		
Controls	n	$\bar{x}$	S.D.	Range	$\bar{x}$	S.D.	Range
I & II	35	6.1	5.4	0.5-25.0	0.2	0.2	0-1.3
IV & V	72	8.4	5.8	0.7-29.0	0.3	0.4	0-2.2
"p" Value		$.05 > p > .01$			< 0.5		
Patients							
I & II	43	6.1	4.8	Tr.*-21.0	0.2	0.2	0-0.9
IV & V	81	8.8	6.1	0.7-42.0	0.3	0.3	0-2.6
"p" Value		$.05 > p > .01$			NSD**		

*Trace Amounts
**No significant difference at .05 level

Total DDT residues in cancer cases and others finding no significant differences (Radomski, Deichman, Ray [4], Dacre and Jennings [5], Casarette, Fryer, Yauger and Klemmer [6], Hoffman, Adler, Fishbein and Bauer [7]. This study does not support a carcinogenic potential of incidental exposure to DDT and dieldrin in this area in these cancers and the data emphasizes that in future case control studies of persistent environmental pollutants, due consideration will have to be given to the more important geographic and demographic variables particularly the socio-economic variable.

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EPSTEIN (U.S.A.)

The observations presented by the speaker, though of great interest in themselves, do not appear to support his contention that they are not consistent with the relationship of DDT and dieldrin to human carcinogenesis. The studies reported were designed to compare residues of these pesticides in relatively small human populations with and without a variety of malignant neoplasms. The studies clearly were not designed to investigate the human carcinogenicity of dieldrin and DDT. To achieve the latter objective, large populations with widely varying body burdens of these pesticides would have to be followed for several decades. Such studies, while feasible would be clearly complicated by the ubiquity of environmental and human contamination with both these persistant pesticides.

DAVIES (U.S.A.) *(Editorial note: written answer submitted later)*

I agree with the comments from Dr. S.S. Epstein. The observations though small at least might be considered reassuring with regard to DDT and human carcinogenesis. All that can be said is that in the sample size studied there was no increase in residues of these pesticides in patients with these malignant neoplasms.

As suggested by Dr. Epstein, prospective studies in high and low exposure groups with sufficient years of exposure will be necessary to settle the issue conclusively. With the better understanding of the epidemiology of the DDT residue in the United States and with special categorization of special factors such as social class it is feasible to do retrospective studies on populations of fairly large size. This would throw some light on the issue but again one can foresee the interpretative phenomena which would complicate such studies; these are: (1) the concept of inducers and promoters in carcinogenesis and the theory that two separate insults are required for human carcinogenesis, and (2) is the issue that already, at least in Dade County, insofar as DDT residues are concerned these are declining in the population and no DDT has been identified in air samples taken in this area for the last two years.

DISCUSSION

(Editorial note: the paper was presented by J.A. Santolucito (U.S.A who answered the questions during the discussion).

WASSERMAN (Israel)

It seens valuable to compare levels of OCI in lipids extracted from the tumoral and presumably health tissues from the same patients for an appropriate comparison of the storage process.

SANTOLUCITO (U.S.A.)

Such comparisons would of course be interesting. However, the levels of organo chlorine insecticides in adipose tissue have been shown to reflect body burdens in relation to exposure rate. Thus, while tumoral tissue concentrations of the insecticides are not known, the observation still holds that body burdens of DDT and dieldrin were not different between cancer and matched non-cancer cases.

OLOFFS (Canada)

I think there is a misunderstanding between the speaker and Dr. Wassermann. According to my interpretation of the paper by Dr. Davies et al., his group did not look at adipose tissue obtained from the cancerous areas, merely at adipose tissue from another area of the body. Consequently, Dr. Wassermann's question is irrelevant, although the problem he raised is of very great interest and significance.

CHAMBERS (Ireland)

I notice that the results were presented for wet weight of tissue. I believe that by now we should be attempting to produce our results as an expression of the dry weight of the tissue as a matter of normal technique. This may be the case especially when tissues have been taken during surgery when there can be great changes in wet weight during storage for example.

SANTOLUCITO (U.S.A.)

Your point is well taken provided, of course, that the dehydration process did not result in losses of the material to be measured. Implicit in the wet weight methodology is that care must be taken to prevent or minimize water loss.

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TIERUNTERSUCHUNGEN
ANIMAL STUDIES
ETUDES SUR LES ANIMAUX
STUDI SUGLI ANIMALI
ONDERZOEKINGEN BIJ DIEREN

Vorsitzender - Chairman - Président - Presidente - Voorzitter

L. COIN (France)

ANIMAL MODELS FOR HUMAN DISEASE

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ABSTRACT

Epidemiology and animal toxicology together yield health effects data which serve as the guidelines for formulation of reasonable air quality standards. Epidemiological studies suggest human health effects of pollutants in the ambient air, but cannot provide direct "cause-and-effect" proof. Community studies must cope with a host of complex covariates, are restricted to a limited range of exposures, and often provide information too late for preventive measures. Toxicological studies in laboratory animals provide the opportunity to employ controlled conditions, to utilize a wide range of pollutant exposures, and to predict potential hazards. These two disciplines complement one another in providing information suggesting the possibility of a cause and effect relationship which can then be used to set standards and do prospective studies. This paper will be directed toward the description, characterization, and evaluation of currently available animal models which have been or could be used as biological indicators of adverse human health effects of environmental agents.

New technological innovations often produce a change in type and quantity of pollutants. A variety of new substances could be produced and distributed into the environment before their effects on health are fully known. It is only through close coordination between human and animal studies that environmental

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science can keep abreast of this rapidly changing challenge to our environment.

Since it is not possible to present a detailed description of all available animal models in the time allotted, this paper will illustrate the contributions which some models have made to our overall understanding of environmental toxicology and indicate how these models can relate to epidemiological studies.

Introduction

The synergistic interaction of epidemiologists, clinicians and animal toxicologists has yielded an impressive body of scientific information which points to the inescapable conclusion that contamination of community air with environmental pollutants can produce profound health consequences. While epidemiologic studies suggest human health effects of pollutants in ambient air, such community studies usually cannot provide "cause-and-effect" proof since they must cope with a host of complex covariates. Furthermore, such studies are restricted to a limited range of exposure, and often provide information too late for preventive measures.

Toxicological studies using animal models provide the opportunity 1) to control some covariates, 2) to utilize a wide range of pollutant exposures, 3) to evaluate a possible threshold for production of adverse health effects, 4) to study effects of single agents as well as the interaction of several pollutants, and 5) to study the basic mechanism of interaction between pollutant and the living system. Most importantly, such studies permit animals to be exposed to newly-introduced products and serve as indicators of health effects before humans are subjected to environmental exposure to new agents.

What is the best choice of animal models? The selection of the animal model must reflect the need of the researcher. Some researchers prefer normal random bred healthy animals while other studies are conducted with modified, but healthy, animals, such as inbred, specific pathogen-free or germ-free. In environmental health studies, specialized animals with either induced or spontaneous specific diseases, such as emphysema, genetic or infectious, may more sensitively reflect health hazards of selected high risk individuals within the human population. Pregnant animals offer the toxicologist the opportunity to study not only the potential effects on the parents but extend the study to other generations. The essential quality of a good animal model is its ability to reliably and sensitively predict a disease process in man.

There are two general approaches that can be employed utilizing animal models. First are those studies that have an immediate applied goal centering around the detection of adverse health effects. The parameters indicating injury may vary from mortality to very subtle cellular and subcellular responses. This type of model seeks to identify the target organ or function which is the most sensitive to the assault.

The second approach has a different goal which is to study the mechanism of the observed toxic effect. These studies could involve alteration in structure or function of organs or individual cells or subcellular functions. In this type of study it must be recognized that while one seeks out a particular target one tends to lose identity with normal physiologic influence of the whole animal.

With all animal studies there are certain biological factors which must be considered before extrapolating the observation to human population. Questions arise such as how to consider the life span of the species and what segment of the animal's life to test. It can be misleading to try to extrapolate years of animal exposure to years of human exposure. Animals may not live long enough to develop diseases that require a long latent period. What dose in the laboratory animal corresponds to what dose in man? Does animal response always indicate danger to man? Does the animal physically and metabolically handle exogenous agents in a manner similar to man? The problem of small sample size must also be considered. An adverse response with a relatively low incidence (i.e. 1:1000) could be considered highly undesirable in the community, although the usual toxicologic study which employs 100 or fewer animals per treatment group would miss this effect. Table 1 lists other more obvious problems that require consideration.

Table 1

Cautions When Using Animal Models for Predicting Effect in Man

1. Species differences.
    a. metabolic
    b. longevity
    c. anatomic
    d. physiologic
    e. genetic
2. State of health.
3. Nutritional requirements.
4. Route and schedule of administration.
5. Small sample size.
6. Endogenous flora.
7. Micro-environment of test animals.
8. Spontaneous disease.

Animal models have served to predict, substantiate or detect the effects of environmental pollutants on alterations in lung function and structure, resistance to disease, behavioral changes, and cancer. Obviously this presentation cannot review all the animal models that have contributed to our knowledge of environmental toxicology, but I would like to discuss one in more detail as an example.

There is good agreement between epidemiologic and animal studies concerning the effect of polluted air on the infectious disease processes, and increasing emphasis is placed on the role played by pollutants in the enhancement of morbidity from respiratory infections.

A model system which has been used in this type of study is to expose animals to a pollutant either before or after a respiratory challenge with an infectious microorganism. Table 2 depicts in outline form the effect of the pollutant on increasing susceptibility which can be measured by effect on (1) mortality rates, (2) survival time,

Table 2

Infectivity Model
Parameters of Response

A. Whole Animals
    1. mortality rates
    2. survival rates
    3. pathology
B. Host Defenses
    1. Pulmonary Defense Cells
        Numbers
        Viability
        Phagocytic Capability
        Bacteriocidal Activity
        Hydrolytic Enzymes Activity
        Clearance Kinetics
    2. Immunological
        Cell-mediated
        Immunoglobulins
        Antibody Levels

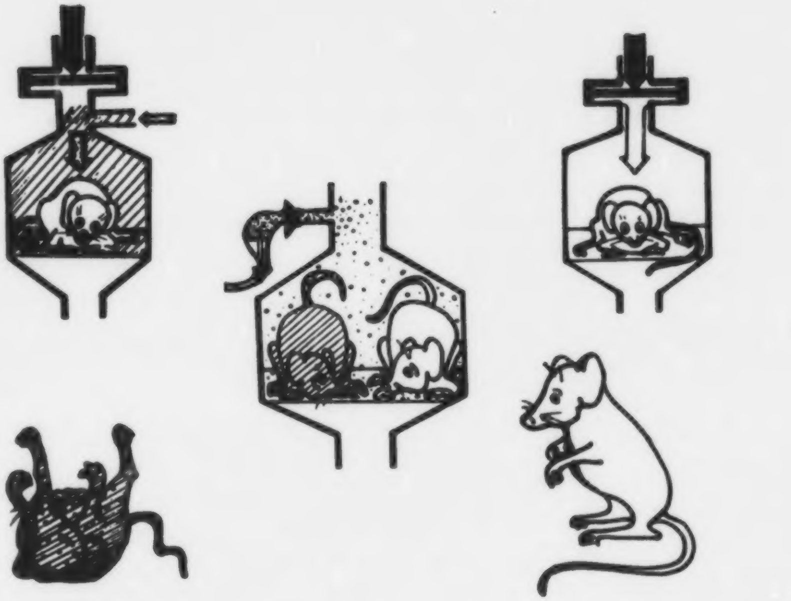

Figure 1. A model for determining the effect of a test substance on host's defenses. Animals are exposed in separate chambers to either the test substance or to clean air. They are then combined and exposed to an aerosol of viable microorganisms. The animals are then separated and various health indicators, such as mortality, are studied.

(3) phagocytic capability, (4) bacteriocidal activity, (5) hydrolytic enzymes activity, (6) inflamatory response, and (7) alteration in immune competence. Figure 1 is a schematic diagram of this model system. Increased susceptibility to a number of microorganisms such as Streptococci, Klebsiella, Diplococcus, and Influenza virus in mice, guinea pigs, hamsters, and monkeys have been associated with exposure to such environmental contaminants as O_3, NO_2, SO_2, Mn, Ni, Pb, auto-exhaust, and dust (Coffin [1], Ehrlich [2], Fenters [3], Ardelean [4], Maigetter [5], Hemphill [6], Port [7], Coffin [8]). Since this effect was first noted the model system has been expanded to study the mechanism of the observed adverse effects (Gardner [9], Coffin [10], Gardner [11], Goldstein [12]).

At the present time other animal models are being developed which may be applicable to community studies. Models which study the effect of pollutants on humoral and cellular immunity; teratogenesis, mutagenesis and post-natal development; acceleration of aging, shortening of life span; and tolerance to the pollutant.

Within our society there exist certain high-risk individuals who are predisposed to the adverse effects of pollutants. Research on the interaction of environmental pollutants and established human disease is currently inhibited because so little of the needed research can be done on human subjects. But many diseases similar to those in humans occur naturally in animals or may be deliberately induced on a predictable basis. Some natural diseases of animals that mimic human diseases are emphysema, autoimmunity, atherosclerosis and cataracts. A more complete list of such models with references has been published (Jones [13]). Obviously these animal models offer many advantages in the establishment of controlled studies of the interaction of environmental factors and pre-existing disease states.

In conclusion it would be appropriate to re-emphasize the importance of communication between the investigators using animals and those with human subjects. By sharing ideas certain guidelines concerning priorities, experimental design, and the practical application of the data will be developed which will mutually aid each group in their research. Such animal studies, coupled with human studies, provide a noble example of a partnership between two disciplines seeking to improve human existence.

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DISCUSSION

AUBERT (France)

I should like to ask the author which animal model he uses or recommends in studying the phenomena of concentrations of toxic chemicals, which increase the further one progresses along the natural biological chains and which, beginning with infinitesimally small doses, may end in levels hazardous to the final consumer - man.

Some years ago now we presented the findings of a study of these phenomena in respect of the ocean, using an original method whereby the experimental marine trophodynamic basins recreated, in the laboratory, the main natural biocenoses: the pelagic chain, the benthic chain and the molluscan or crustacean neritic chains.

In fact we describe our method in the paper "Les chaines trophodynamiques marines experimentales: méthode d'étude des effets pathologiques consecutifs aux pollutions chimiques" presented at this Symposium, to which reference may be made.

My question is thus as follows: did the author use this method? Is he aware of similar findings by groups other than our own?

GARDNER (U.S.A.)

No, I have not personally used such an elaborate system as you and your colleague have described. Your model system appears to systematically investigate the fate and potential toxicity of chemicals as they pass through various biological systems in hope to mimic what is occurring naturally. There are, of course, many studies designed to follow the transferral of pesticides via the food chain. These studies indicate that many factors can directly effect the persistence, metabolism and movement of such chemical agents through the ecosystem; for example, the quality of water, abundance of plants and animals, water temperature, species variability, etc. In order to obtain the most information from your model systems, all of these variables need close control. I will be looking forward to seeing some of the toxicological data generated through the use of your model system.

WASSERMAN (Israel)

Toxic effects in the food chain are studied with the aim of detecting the most susceptible species, whose damage or distruction would disturb the ecosystem and in the last instance the man.

The Ministry of Interior - Wildlife Research Group - is developing such studies to organocholrine insecticides movement in the ecosystem. Other aspects related towards this topic will be reported in the Symposium on Biotoxicity which will be held on July 1, 1974 at the Environmental Medicine and Biology Congress in Paris.

BITTEL (France)

In my opinion, consideration should be given to the phenomena of concentration not on only the level of organisms and tissues, but also on the sub-cellular level.

GARDNER (U.S.A.)

I think Dr. Bittel's point is a good one; that is, it is desirable to use the most sensitive techniques we have available for determining the concentration at the target site. In many cases one may have to start at the tissue level but hopefully, a more sensitive parameter would be developed to allow one to study sub-cellular localization.

STARA (U.S.A.)

I believe that the previous questions have considered another aspect of animal effects. There are two issues here. The first is the use of animal models in public health application i.e. toxicologic investigations to evaluate and predict potential human health effects. The second is the studies concerned with uptake of hazardous materials in various animal species themselves resulting in a potential distruction of a species and a disruption of an ecological chain. We must note this difference. I believe that Dr. Gardner addressed himself to the first aspect of toxicology.

GARDNER (U.S.A.)

I would like to add that although there are many different applications for animal models in research, it was unrealistic for me to try to review and discuss all those used in environmental toxicology. With each research goal, the animal model must reflect the need of the researcher. These needs vary greatly. I devoted my time to those animal models that mimic human diseases and can thus be used for predicting possible adverse effects resulting from environmental pollution.

DIE TUMORERZEUGENDE WIRKUNG FASERFÖRMIGER STÄUBE

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KURZFASSUNG

Aus früheren tierexperimentellen Untersuchungen mit verschiedenen faserförmigen und körnigen Stäuben wurde geschlossen, dass die kanzerogene Wirkung von Asbest an die Faserform gebunden ist und nicht an seine chemische Zusammensetzung. Weitere Versuche mit UICC-Chrysotil und sehr feinen Glasfasern an Wistar-Ratten ergaben, dass nach intraperitonealer Injektion von nur 2 mg dieser faserförmigen Stäube Tumoren entstanden. Bei dieser Dosis bildete sich keine deutliche Fibrose. Histologisch handelte es sich bei den Tumoren meist um sarkomatöse Mesotheliome.

Die tierexperimentellen Befunde führen zu dem Schluss, dass die Dosis-Wirkungsbeziehungen bei der Tumorinduktion durch faserförmige Stäube von vier Faktoren abhängig sind: Faserzahl, Faserdurchmesser, Faserlänge und Verweildauer am Ort der Wirkung. Es muss damit gerechnet werden, dass extrem feine Glasfasern auch beim Menschen zu Tumoren führen, da die Kanzerogenität von Asbest beim Menschen erwiesen ist, und die Tumoren, die nach intraperitonealer Injektion von Asbest und von Glasfasern bei der Ratte diagnostiziert wurden, sich nicht voneinander unterschieden.

Der grösste wirksame Faserdurchmesser wird auf 1 bis 3 µm geschätzt. Glasfasern mit einem Durchmesser von <5 µm werden in Deutschland erst seit 1961 hergestellt. Mit dem Auftreten von Mesotheliomen beim Menschen ist frühestens 20 Jahre nach

Beginn der Inhalation extrem feiner Fasern zu rechnen. Der Beweis für die Übertragbarkeit der tierexperimentellen Ergebnisse auf die Humanpathologie wird daher kaum vor 1985 möglich sein.

ABSTRACT

Earlier animal experiments using various fibrous and granular dusts led to the conclusion that the carcinogenic effect of asbestos is linked to its fibrous form and not to its chemical composition. Further experiments on Wistar rats with UICC-Chrysotil and very fine glass fibres revealed that tumours developed after intraperitoneal injection of only 2mg of these fibrous dusts. This dose did not cause any clearly-defined fibrosis. Histologically, the tumours were in most cases sarcomatous mesotheliomas.

The results of animal experiments lead to the conclusion that, when tumours are induced by fibrous dusts, the dose-effect relationship depends on four factors: number, diameter and length of the fibres and length of contact with the affected area. It must be expected that extremely fine glass fibres can also cause tumours in humans, as the carcinogenic effect on humans of asbestos has been established, and the tumours diagnosed in rats after the intraperitoneal injection of asbestos and glass fibres did not differ from each other.

The largest effective fibre diameter is estimated at 1 to 3 µm. Glass fibres with a diameter of < 5 µm have only been manufactured in Germany since 1961. The appearance of mesotheliomas in humans can be expected at the earliest 20 years after the initial inhalation of extremely fine fibres. Thus until 1985 it will hardly be possible to prove whether the results of animal experiments can be applied to human pathology.

Die tumorerzeugende Wirkung von Asbest ist seit etwa 40 Jahren bekannt. In letzter Zeit wurden auch andere faserige Stäube im Tierexperiment auf ihre Kanzerogenität untersucht. STANTON und WRENCH (1) berichteten, dass nach intrapleuraler Implantation von sehr feinen Glasfasern bei Ratten Tumoren entstanden waren. Körnige Glaspartikeln führten dagegen in einer signifikant niedrigen Häufigkeit zu Tumoren. POTT und FRIEDRICHS (2) kamen zu dem Ergebnis, dass nach intraperitonealer Injektion von 100 mg Glasfasern bei Ratten ebenfalls Tumoren entstanden. Auch faserförmiges Magnesiumhydroxid mit der mineralogischen Bezeichnung Nemalith wirkte im gleichen Versuchsmodell kanzerogen. Demgegenüber induzierten körnige Mineralstäube, wie Biotit, Hämatit, Sanidin und Talk keine oder nur vereinzelte Tumoren.

Bei Weiterführung unserer Tierversuche haben wir wiederum Glasfasern benutzt, da mit diesem Material ein Modellstaub gegeben war, an dem die Bestimmung der Längen- und Durchmesserverteilung des Faserkollektivs mit grosser Genauigkeit möglich ist. Ihr weiterer Vorteil gegenüber den Mineralstäuben besteht darin, dass sie nicht wie z.B. Asbest durch mineralische Begleitsubstanzen verunreinigt sind. Die Grössenverteilung der benutzten Glasfasern ist in den Abbildungen 1 und 2 dargestellt. Hiernach besteht die feinere Glaswolle MN 104 aus Fasern, die zu 50 % dünner als 0,2 Mikrometer bzw. kürzer als 11 Mikrometer sind. Bei der groberen Glaswolle MN 112 sind demgegenüber über 50 % der Fasern dünner als 1 Mikrometer bzw. kürzer als 28 Mikrometer. Die Stäube wurden in 2 ml 0,9 %iger NaCl-Lösung suspendiert und Wistar-Ratten intraperitoneal injiziert. Die Versuchsergebnisse sind in der Tabelle 1 zusammengefasst. Nach einem Beobachtungszeitraum von 24 Monaten zeigt sich, dass bei dem Glasfasertyp MN 104 eine Dosis-Wirkung-Beziehung besteht. Die angegebene Tumorhäufigkeit je Tiergruppe ist jedoch nicht als endgültig anzusehen, da ein Teil der Tiere noch lebt. Ausserdem ist beim Vergleich zwischen der Glasfaser MN 104 und dem Amphibol-Asbest Krokydolith zu erkennen, dass die Tumorraten bei gleicher Dosis ähnlich sind. Die kanzerogene Wirkung der Glaswolle MN 112 scheint demgegenüber der der MN 104-Faser niedriger zu sein. Die histologische Diagnose der induzierten Tumoren ergab, dass es sich in fast 90 % der Fälle um Mesotheliome handelt. Die übrigen wurden als

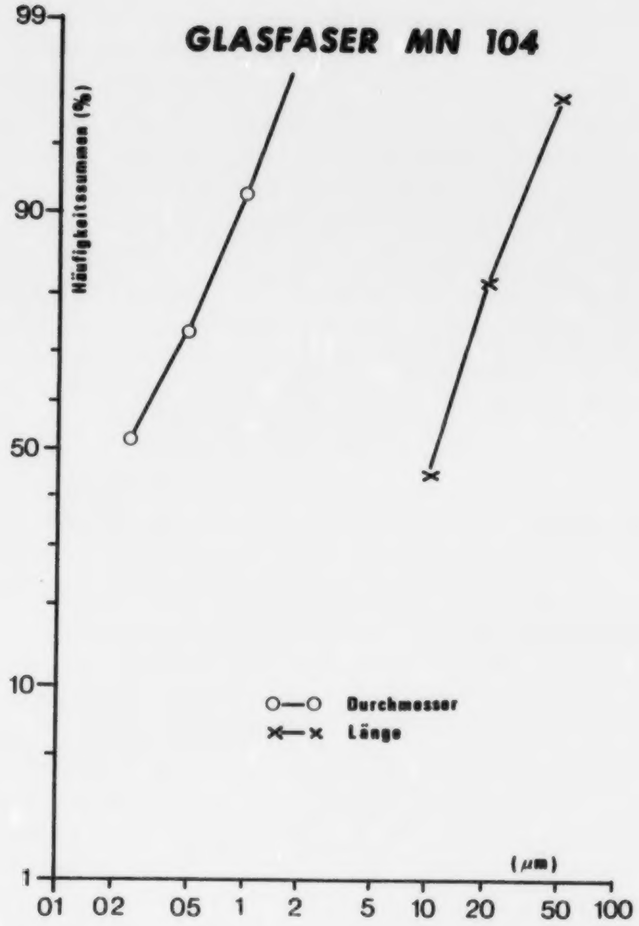

Abbildung 1: Häufigkeitsverteilung von Länge und Durchmesser der Glasfaser MN 104

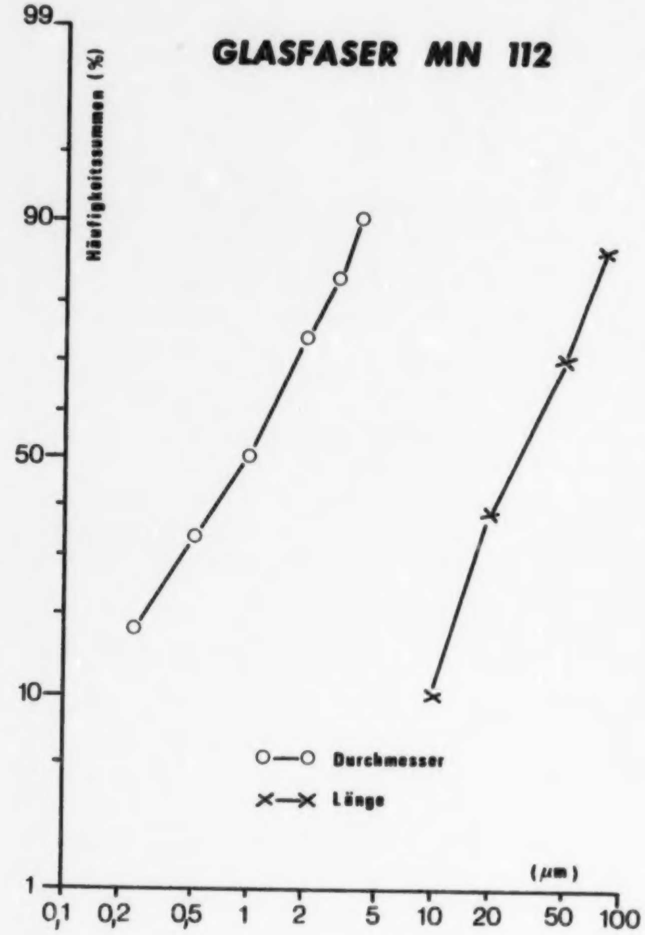

Abbildung 2: Häufigkeitsverteilung von Länge und Durchmesser der Glasfaser MN 112

Tumors after intraperitoneal injection of fibrous glass, crocidolite and corundum in rats
(unfinished experiment about 24 months)

Dust	Dose mg i.p. in 2 ml saline	Number of rats at the start	After 24 months		Histol. diagnosis					Survival time of first rat with tum. days
			surviving rats	% of rats with tum.	Mesothe-lioma	Spindle-c. sarc.	Polym. c. sarc.	Carc.	Retic. c. sarc.	
Fibreglass MN 104	2	80	38	10.0	7	1	-	-	1*	576
"	10	80	21	33.8	23	3	-	1	-	210
"	2 x 25	80	-	68.8	47	6	2	-	2*	194
Fibreglass MN 112	20	40	9	27.5	10	1	-	-	-	390
Crocidolite UICC	2	40	17	10.0	4	-	-	-	2*	452
Corundum	2 x 25	40	25	2.5	1	-	-	-	1*	545

* Spontaneous tumor, not valued

Spindelzellsarkome oder polymorphzellige Sarkome eingestuft. Versuche mit Mäusen ergaben zum Teil eine niedrigere Tumorrate nach intraperitonealer Injektion faserförmiger Stäube. Bei Goldhamstern und Meerschweinchen wurde bisher kein Tumor gefunden, weder nach Injektion von Chrysotil noch von Glasfasern.

Aufgrund unserer inzwischen umfangreichen Versuchsreihen ergibt sich, dass die tumorinduzierende Eigenschaft faserförmiger Stäube massgeblich durch die spezielle Partikelform, nicht dagegen durch die chemische Zusammensetzung bestimmt wird. Wir befinden uns mit dieser Schlussfolgerung in Uebereinstimmung sowohl mit STANTON und WRENCH (1) als auch mit WAGNER, BERRY und TIMBRELL (3).

Die Dosis-Wirkung-Beziehung für die Tumorinduktion ist nach dieser Hypothese vorwiegend abhängig von der Faserzahl, der Faserlänge, dem Faserdurchmesser und von der Verweildauer der Faser am Ort der Wirkung.

Eine Uebertragbarkeit der Ergebnisse von Tierversuchen auf den Menschen ist nicht ohne weiteres zulässig, denn alle hier benutzten Applikationsverfahren, nämlich die intraperitoneale und intrapleurale Injektion und auch die intrapleurale Implantation müssen als unphysiologisch angesprochen werden. Da jedoch die kanzerogene Wirkung von Asbest auf den Menschen bekannt ist und da die im Tierversuch mit Asbest und anderen faserförmigen Stäuben induzierten Tumoren sich nicht voneinander unterscheiden, erscheint der Hinweis auf ein mögliches Gesundheitsrisiko für den Menschen berechtigt.

Die wirksame Dosis ist unter anderem abhängig von der Konzentration atembarer Fasern. Die nachfolgend zitierten Konzentrationsangaben über Fasern in der Aussenluft sind unter dem Vorbehalt zu werten, dass der Begriff "Faser" hinsichtlich seiner Kenngrösse L/D international bislang nicht einheitlich definiert ist. So fand THAER (4) im Raum Frankfurt in der Aussenluft 8000 Fasern/m^3. SPURNY (5) ermittelte an verschiedenen Messstellen zwischen 1000 und 100.000 Fasern/m^3. Eigene Messungen in Düsseldorf und Gelsenkirchen erbrachten Konzentrationen zwischen 1000 und 8000 Fasern/m^3 bei einem gewählten L/D-Verhältnis von 5 : 1. Bei den erwähnten Messprogrammen ist eine Unterscheidung hinsichtlich der stofflichen Zusammensetzung bisher nicht erfolgt.

Die Konzentration ist demgegenüber an Arbeitsplätzen von Betrieben, in denen Fasern hergestellt oder verarbeitet werden, vermutlich wesentlich höher. Am Beispiel der Glasfaserindustrie muss ausserdem darauf hingewiesen werden, dass seit ca. 15 Jahren zunehmend feinere Fasern hergestellt werden (6). Der Anteil der atembaren Fasern steigt dementsprechend an.

Zusammenfassend ist festzuhalten, dass aufgrund einer Vielzahl von Versuchen mit Ratten die Kanzerogenität faserförmiger Stäube nachgewiesen wurde. Hieraus wird ein potentielles Gesundheitsrisiko für den Menschen abgeleitet, dessen Umfang erst durch zukünftige epidemiologische Untersuchungen ermittelt werden kann.

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DISKUSSION

SCHLATTER (Schweiz)

Bestehen Anhaltspunkte dafür, dass faserförmige Stäube in der Nahrung zu Tumoren führen können?

FRIEDRICHS (Bundesrepublik Deutschland)

Eine Tumorinduktion durch oral aufgenommene Fasern lässt sich nicht ausschliessen, uns sind jedoch keine Befunde bekannt, die eine solche Wirkung wahrscheinlich machen.

SCHLIPKÖTER (Bundesrepublik Deutschland)

1. Das Auftreten von bösartigen Magentumoren ist bei peroraler Applikation nicht bekannt, jedoch nach Inhalation sind Magentumore beobachtet worden.

2. Die kanzerogene Wirkung der Fasern ist tierspezifisch. Da jedoch Asbest bei Mensch und Ratte bzw. Mäuse kanzerogen wirken und da das gleiche mit Glasfasern bei Ratten und Mäusen beobachtet wurde, muss in der Zukunft faserförmigen Stäuben grössere Aufmerksamkeit geschenkt werden.

3. Die von Pott und Mitarbeitern beobachteten Tumore waren nicht nur Mesotheliome sondern es wurden auch Sarkome sowie andere bösartige Tumore diagnostiziert.

OLOFFS (Canada)

Können Sie sich die negativen Resultate mit Goldhamstern und Meerschweinchen erklären?

FRIEDRICHS (Bundesrepublik Deutschland)

Die negativen Befunde bei Goldhamstern und Meerschweinchen können wir vorläufig nur mit dem allgemeinen Hinweis darauf erklären, dass die Empfindlichkeit gegenüber Substanzen in vielen Fällen grosse artspezifische Unterschiede aufweist. Möglicherweise sind die Unterschiede im Immunverhalten, die insbesondere zwischen Meerschweinchen und Ratten bestehen, von Bedeutung.

REEVES (U.S.A.)

Ich glaube, zwei verschiedene Prinzipien können bei der Entstehung von Lungenkrebs und pleuralen Mesotheliomen nach Asbesteinatmung eine Rolle spielen Das Lungenkarzinom scheint eine relativ hohe Spezifität als Agens zu haben; meines Wissens hat bisher noch niemand die zuletztgenannte Läsion mit nicht-asbesthaltigen faserförmigen Stäuben hervorbringen können. Paul Gross hat sogar nachgewiesen, dass Glasfasern nach Einatmung in dieser Beziehung unwirksam waren. Andererseits hat das Mesotheliom eine viel niedrigere Agens-Spezifität und kann durchaus von der Geometrie der Stäube abhängen, da sowohl Asbestfasern als auch Glasfasern mit Erfolg von Stanton & Wrench verwendet worden sind.

Weiter glaube ich, dass die offensichtliche Spezifität bei der karzinogenen Wirkung von Asbest mit der Bereitschaft der Gasttiere verbunden ist, ferrogene Körper zu entwickeln. Die Arten mit der ausgeprägtesten Fähigkeit, ferrogene Körper zu entwickeln (z.B. Meerschweinchen), sind anscheinend immun gegen eine Asbest-karzinogenese, während Arten mit einer entsprechend geringen Fähigkeit hierzu (z.B. Ratten) am anfälligsten sind. Die Bildung ferrogener Körper ist möglicherweise eine Schutzmassnahme des Organismus.

FRIEDRICHS (Bundesrepublik Deutschland)

Sicherlich muss daran gedacht werden, dass die Entwicklung des Asbest-induzierten Bronchialkarzinoms von anderen Eigenschaften des Asbests hervorgerufen wird als die Entwicklung des Asbest-induzierten Mesothelioms. Die Untersuchungen von Gross erbringen jedoch unseres Erachtens nicht den Beweis dafür, dass Glasfasern nicht zu einem Bronchialkarzinom führen können.

TERATOGENIC, MUTAGENIC, AND CARCINOGENIC EFFECTS OF INSECTICIDES

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ABSTRACT

There is increasing concern over the possible toxicological hazards posed by a spectrum of environmental chemicals including industrial pollutants, pesticides, food additives and drugs, either considered singly as a class or specific agent or in combination. A number of insecticides, based on aspects of their ubiquity, persistence, presence and/or concentration in the food chain as well as their biological and toxicological properties, constitute a major source of potential environmental hazard to mammal species including man.

However, there exists considerable disagreement concerning the scientific assessment of effects (notably teratogenic, mutagenic and carcinogenic) of these agents, primarily because of the variety of testing procedures employed within the three major areas of toxicity, hence complicating comparisons within any one area; complexity of unambiguous recognition and interpretation of the toxic event and finally the extrapolation and relevance of experimental laboratory findings to man.

To permit a more orderly assessment of the above effects the possible interrelationships between teratogenicity, mutagenicity and carcinogenicity will be cited as well as the most salient features of the respective testing procedures employed

with concomittant clarification of their principles, problems and interpretations.

The teratogenic, mutagenic and carcinogenic insecticides that will be discussed include the following:

chlorinated hydrocarbons (DDT and metabolites); cyclodienes (dieldrin, aldrin and endrin); miscellaneous chlorinated insecticides (benzene hexachloride and Mirex); organo-phosphorus derivatives (dichlorvos (DDVP), trichlorphon, malathion, parathion and methyl parathion) and carbamates (carbaryl).

The major objectives of this presentation is to highlight the principal factors in the assessment of the teratogenicity, mutagenicity, and carcinogenicity of a number of the commercially important chlorinated insecticides, and secondarily, to consider some of the consequences of their increasing replacement by a number of organophosphorus and carbamate insecticides.

The complexity of unambiguous recognition of teratogenic, mutagenic, and carcinogenic events is well recognized. Table 1 illustrates a number of the principal factors influencing carcinogenicity testing. The cogent factors that must be vigorously assessed in carcinogenesis investigations include: route of administration, dosage, frequency of exposure; species, strain and sex of test animal; age of animal at the start of the test; basic diet and possible dietary contaminants (e.g., pesticides, metals - mercury, cadmium, lead, arsenic and selenium - mycotoxins, PCB's, estrogens, synergists; levels which can change based on geographical and seasonal considerations), immunological status of the animal and duration of the experiment; homogeneity of the test material, possible impurities in air, water, bedding (pentachlorophenol, trace metals, etc.), housing conditions (crowding stress, intercurrent disease, and drug therapy). It should be noted that the selection of doses, frequency of exposure, and duration of test is still a matter of controversy.

No less controversial, and often conflicting, are factors concerning the final assessment of carcinogen testing as exemplified by Table 2. There is by no means common agreement as to assessing either spontaneous or induced tumor incidence in laboratory animals. Diagnostic criteria applied to lesions and thoroughness of autopsy,

TABLE 1

PRINCIPAL FACTORS INFLUENCING CARCINOGENICITY TESTING

1. Route of Administration, Dosage, and Frequencing of Exposure.
2. Homogeneity of Test Compound (Purified Material; Commercial Prep; Mixture of Isomers; Trace Contaminants).
3. Species, Strain, and Sex of Test Animals.
4. Age of Animal at Start of the Test.
5. Immunological Status.
6. Diet, Nutrition, and Interactions.
7. Possible Diet Contaminants (Pesticides, Heavy Metals, Trace Elements, Mycotoxins, PCB's, Estrogens, Enzyme Inducers).
8. Pollutants and Impurities in Air Water, Bedding (Pentachlorophenol, etc.).
9. Duration of the Experiments.
10. Housing Conditions (Crowding Stress; Intercurrent Disease; Drug Therapy).

TABLE 2

FINAL ASSESSMENT OF CARCINOGEN TESTING

1. Analysis of Histological, Cytological and Electron Microscopic Aspects of Pre-Malignant of Malignant Change.
2. Range of Precancerous and/or Hyperplastic Lesions to Unequivocal Malignant Change.
3. Confusion of Terminology ("Benign Tumor", "Hepatoma", "Nodular Hyperplasia", "Benign Hepatoma").
4. Extrapolation from Experimental Results to Determination of Safety Factor. (No Effect Level ?).

with concomittant differentiation of benign and malignant tumors, hyperplasia, metaplasia, dysplasia, and neoplasia being assuredly fundamental in any valid assessment of carcinogenicity testing.

The difficulty in the assessment of carcinogenicity testing is best exemplified in the case of DDT. Table 3 summarizes the results of 18 studies with DDT involving a broad range of animal species, strains, dosages, frequency of dosages, and duration of tests. Of special note is the lack of uniformity of test material from one study to another, which ranges from p,p-DDT of no stated purity, to technical material which can contain a mixture of at least 16 components, ranging from the isomeric DDT's and DDD's to varying levels of synthetic precursors and degradation products as shown in Table 4. Several multigenerational studies with DDT have also been reported.

Studies in 5 generations from the inbred BALB/c mouse strain were carried out by Kemeny and Tarjan (1) with dietary DDT at a level of 2.8 - 3.0 ppm. Chemical analysis of this basic diet showed the presence of 0.2 to 0.4 ppm DDT as contaminant, possibly accounting for the occurence of the few observed leukemia cases in controls, as well as to the level of 12.4% of DDT fed animals with leukemia and 24.7% with tumors.

Tumor and leukemia incidences, generation by generation calculated by Kay (2) and noted by Terracini (3) did not increase with succeeding generations giving rise to the question as to whether the age differences in the number of mice in generation groups may have marked the outcome (Table 5).

Tomatis and co-workers (4) have been investigating the multigenerational effect on technical DDT (70 - 78% p,p - DDT, 20% o,p -

TABLE 3

SUMMARY OF RESULTS OF LONG-TERM FEEDING EXPERIMENTS WITH DDT

SPECIES AND STRAIN	NUMBER OF ANIMALS		RANGE OF INTAKES (MG/KG/DAY)	LENGTH OF TREATMENT (MONTHS)	DOSE LEVELS[1]	EVIDENCE OF CARCINOGENICITY	DETAILS DDT USED	REFERENCE
	TREATED	CONTROLS						
Rats, Osborne Mendel	192	36	5-40	24	6	Results for each dose level not given. In experimental rats surviving more than 18 months 4/75 with liver tumors (1% in untreated rats) and 11/75 with hyperplastic nodules	Technical	Fitzhugh and Nelson, 1947
Rats	56	14	15-90	12	5	None	Not given	Haag et al. (1950)
Rats	37	20	0.36-36[2]	10-15	4	None	Not given	Cameron and Cheng (1951)
Rats, Carworth	240	80	0.12-1.2	24	4	None	Not given	Treon and Cleveland (1955)
Rats	25	--	0.01[2]	17	1	None	Not given	Klimmer (1955)
Rats, Sherman	75	50	1-2	10	2	None	pp'-DDT	Kimbrough et al. (1964)
Rats, Osborne Mendel	60	60	4	24	2	Increase of lung tumours (8 vs. 2 in controls) not confirmed in other groups given DDT + other pesticides	Not given	Radomski et al. (1965)
Rats, Osborne Mendel	60	60	10	27	2	In expt. 1 group hepatoma and 1 gastrictumour	pp'-DDT	Deichmann et al. (1967)
Rats, Fischer	30	--	30-100[3]	12	1	None	Not given	Weisburger and Weisburger(1968)
Syrian Golden Hamster	115	79	50-120	24	3	None	pp'-DDT	Agthe et al. (1970)
Mice ?	50	--	1.5-15	?	?	4/50 with gastric adenocarcinomas (mice on unbalanced rice diets)	Not given	Smith (1962)
Mice (C57BL6 x C3H anf)F$_1$	36	166	21-46[4]	18	2	11/18♂ and 4/18♀ with hepatoma (vs. 8/79 and 0/87 in controls)	pp'DDT	Innes et al. (1969)
Mice (C57BL/6 x AKR)F$_1$	36	172	21-46[4]	18	2	7/18♂ with hepatoma (5/90 in controls) 6/18♀ with lymphoma (vs. 4/82)	pp'-DDT	Innes et al. (1969)
Mice, BALB/c	683	406	0.4	24	2	18.7% with tumours at various sites and 12.4% with leukemia (vs. 3.2% and 2.5%)	pp'-DDT	Tarján and Kemény (1969)
Mice, CF1	881	224	0.3-37.5	24	5	Increased incidence of hepatoma in ♀ at 0.3 mg/kg/day (46% vs. 22%) and above and in at 1.5 mg/kg/day in F1 generation (14% vs. 4%) Dose-response relationship	Technical	Tomatis et al. (1972)
Trout, Rainbow	?	400	75[5]	15	2	11/35 with gross hepatomas (no tumors in controls)	Not given	Halver et al. (1967)
Trout, Rainbow	?	?	18-75[5]	20	3	8/229 at 18 ppm and 10/196 at 75 ppm with gross hepatomas (2/284 in controls)	Not given	Halver et al. (1967)
Monkeys, Rhesus	22	9	5-5000[5]	90	5	None	Technical or pp'	Durham et al. (1963).

[1] Including dose level 0 of the control group, where it exists.

[2] Stomach tube.

[3] 10 mg/rat five times weekly starting at weaning.

[4] Up to 46 mg/kg/day by stomach tube from day 7 to day 28 of age, then 140 ppm in the diet.

[5] ppm in the diet.

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TABLE 4

COMPOSITION OF TECHNICAL DDT

COMPOUND	SAMPLE 1 (SETTING POINT, 91.2), %	SAMPLE 2 (SETTING POINT, 88.6), %	SAMPLE 3 (SETTING POINT, 91.4), %	SAMPLE 4 (BY-PRODUCT OIL), %
1-Trichloro-2.2-bis (p-chlorophenyl)-ethane (p,p'-DDT)[a]	(a) 66.7, (b) 72.9	(b) 70.5, (c) 63.5[a], (d) 64.5, (e) 67.9	(a) 72.7, (b) 76.7	....
1-Trichloro-2-o-chlorophenyl-2-p-chlorophenylethane (o,p'-DDT)	19.0	(c) 7.9, (d) 15.3, (e) 20.9	11.9[b]	74.8[a]
1,1-Dichloro-2,2-bis-(p-chlorophenyl)-ethane (p,p'-DDD)	0.3	4.0	0.17[d]	....
1,1-Dichloro-2-o-chlorophenyl-2-p-chlorophenylethane (o,p'-DDD)	....	....	0.044	....
2-Trichloro-1-o-chlorophenylathyl p-chlorobenzenesulfonate	0.4	1.85	0.57	0.11
2-Trichloro-1-p-chlorophenylethanol	0.2	....	....	....
Bis-(p-chlorophenyl)-sulfone	0.6	0.1	0.034	....
α-Chloro-α-p-chlorophenylacetamide	....	0.01	0.006	....
α-Chloro-α-o-chlorophenylacetamide	....	0.007	....	....
Chlorobenzene	....	....	....	2.44
p-Dichlorobenzene	....	....	....	0.73
1,1,1,2-Tetrachloro-2-p-chlorophenylethane	....	....	....	+[a]
Sodium p-chlorobenzene-sulfonate	0.02	....	....	....
Ammonium p-chlorobenzene-sulfonate	....	....	0.005	....
Inorganic	0.1[f]	0.04[g]	0.01[h]	....
Unidentified and losses	6.5	5.1	10.6	19.4

[a] Letters in parentheses refer to analytical methods as follows: (a) Isolation from technical DDT, (b) recrystallization from 75 % aqueous ethanol previously satured with p,p'-DDT,(c) fractional crystallization, (d) adsorption analysis and fractional crystallization, (e) isolation, supplemented by cryoscopic analysis on theresidue. [b] This value does not represent all the o,p'-DDT present, as all oily fractions were not exhaustively studies. [c] Miscellaneous fractions containing p,p'-DDT, and p,p'-DDD. [d] Includes 0.06% of p,p'-DDD isolated as such and 0.11% of the corresponding olefin. [e] Isolated as nitro derivative from an oil mixture analyzing for a mixture of $C_6H_4CL_4$and $C_5H_5CL_5$ and representing 2.54% of original material. [f] Qualitative tests for ferric, lead and magnesium carbonates were obtained. [g] Insoluble in boiling 95% ethanol [h] Qualitative tests for ferric, ammonium, halide and sulfate ions were obtained.

TABLE 5

CARCINOMA INCIDENCE AMONG BALB/c MICE FED 2.8 TO 3.0 PPM DDT OVER 5 GENERATIONS (CALCULATED FROM DATA OF TARJAN AND KEMENY (1)

Groups	Age mo	No. of mice	Tumors incidence %			Leukemia incidence %			Total[a] incidence
			Male	Female	Total	Male	Female	Total	
F_1	26	10	10	20	30	10	30	40	70
F_2	22	35	26	23	49	5.7	0	5.7	55
F	18	69	9	22	31	1.5	14.5	16	47
F	15	264	11	13	24	2.7	10.6	13	37
F	11	305	10	20	30	3.3	7.5	11	41
Total		683	11.5	17.5	29	3.1	9.4	12	41
Controls total									
Total	-	406	0.49	2.71	3.20	0.74	1.72	2.46	5.66

[a] Total of male and female, both types of carcinoma.

DDT, 0.5-1.5% p,p - DDD, and 0.5% p,p - DDE) fed to CF-1 inbred mice maintained as specific pathogen-free at dose levels of 2, 10, 50, and 250 ppm. Liver tumors appeared earlier in the F_1 mice fed 50 to 250 ppm. Out of several hundred liver tumors in the DDT-fed groups, only 4 gave metastases to the lung. Non-invasive, non-metastasing liver tumors predominated. Difficulties of differentiating benign liver tumors from hyperplastic nodules were reiterated. There was little apparent evidence between P and F_1, generations, either for all tumors or only liver tumors which represented the only tumor type (among 3 main types) to be dose related.

Confusion persists in the interpretation of the tumorigenicity of aldrin, dieldrin, and endrin. Of four studies (Table 6) dealing with the relationship between exposure to these chemicals and tumor

TABLE 6

ALDRIN, DIELDRIN, AND ENDRIN

AGENT	SPECIES	DOSE	EFFECT	REFERENCE
Aldrin, Dieldrin	Rat (Osborne-Mendel)	0.5, 2, 10, 50 100 or 150 ppm (2 years)	Pulmonary Lymphosarcoma (Enhanced tumor incidence in high survival-low dosage group).	Fitzhugh, O. G., et al Food. Cosmet. Toxicol., 2 (1964) 551
Aldrin, Dieldrin	Mouse (C_3HeB/Fe)	10 ppm (2 years)	Hepatomas, Pulmonary Adenoma, Lympoma, 17%, 18% over-all tumor incidence for Aldrin, Dieldrin	Davis, K. J. and Fitzhugh, Toxicol. Appl. Pharmacol., 4 (1962) 187.
Dieldrin	Rat (Carworth Farm "E" Strain, SPF Conditions)	0.1, 1.0, and 10.0 ppm (2 years)	Non-Tumorigenic	Walker, A. I. T. et al, Toxicol. Appl. Pharmacol., 15 (1969) 343.
Endrin	Rat (Osborne-Mendel)	20, 30, 50 ppm (31 months)	Inactive	Deichmann, W. B., et al, Ind. Med., 39 (1970) 426
Aldrin, Dieldrin	Rat (Osborne-Mendel)	20, 30, 50 ppm (31 months)	Anti-Tumorigenic	Deichmann, W. B., et al Ind. Med., 39 (1970) 426

incidence, it was concluded in two studies by Fitzhugh and co-workers that aldrin and dieldrin were tumorigenic in the rat and mouse. (5, 6).

In a third study involving 2 year oral exposure of rats and dogs, aldrin was found inactive by Walker and co-workers (7). In a fourth study reported by Deichman and co-workers, aldrin and dieldrin were judged to be antitumorigenic and endrin to be inactive in the rar (8).

Walker et al (9, 10) reported neoplastic liver changes in CF-1 mice fed low doses of dieldrin, DDT, β-BHC, γ-BHC, and phenobarbitone on a long-term basis.

Table 7 illustrates the spontaneous tumor experience with Osborne-Mendel Rats of the FDA-strain and its University of Miami substrain. The 1959 spontaneous tumor incidences with the FDA substrain of Osborn-Mendel rats were 22%. Subsequently, the incidence of this strain was much higher, for example reaching 30,44 and 48% in 1970. The highest incidence in aldrin treated rats in the 1952 FDA experiment carried out by Fitzhugh (but not reported until 1964) was 53% at the lowest level of administration, 0.5 ppm aldrin (Table 8). This suggests according to Kay (2) that spontaneous variability might account for the apparent tumorigenicity of aldrin and dieldrin.

Table 9 illustrates the data of Deichman and co-workers (8) and shows that the incidence of tumors in the aldrin and dieldrin-fed rats was much lower at all levels of dosing than the incidence of spontaneous tumors in controls, and this was the basis for the authors evaluation of anti-tumorigenicity for these compounds. However, this conclusion would be reversed and aldrin and dieldrin considered tumorigenic if the control incidence had been as low in

TABLE 7

SPONTANEOUS TUMOR EXPERIENCE WITH OSBORNE-MENDEL RATS[a]
OF THE FDA STRAIN AND ITS UNIVERSITY OF MIAMI SUBSTRAIN /FROM KAY (2)/

INVESTIGATORS AND REFERENCE NOS.	DATE	NO. OF RATS[b]	SURVIVAL % (2 YEARS)	NO. WITH TUMORS	TUMOR INCIDENCE %
		FOOD AND DRUGS ADMINISTRATION			
Fitzhugh et al. (27)	1959	224	55	49	22
Davis & Fitzhugh (28)	1962	50	42	22	44
Davis & Fitzhugh (29)	1963	50	26	24	48
Davis et al. (30)	1964	24	54	8	33
Fitzhugh et al. (31)	1964[b]	17	50	3	18
Hansen et al. (32)	1965	50	38	23	46
Hansen et al. (33)	1966	50	no info.	16	32
Hansen et al. (33)	1966	50	no info.	18	36
Hansen et al. (33)	1966	50	no info.	15	30
Byron et al. (34)	1967	50	42	16	32
Byron et al. (34)	1967	50	36	17	34
		665		211	32
		University of Miami[c]			
Radomski et al. (35)	1965	60	no info.	15	25
Radomski et al. (35)	1965	100	no info.	26	26
Deichmann et al. (36)	1967	60	45	14	23
Deichmann et al. (37)	1970	163	maximum survival 27 months	79	48

---

[a] Starting age weanling or 3 weeks in all cases; diet Purina lab chow except Refs. 27 and 31, ground commercial; Ref. 33 no information. Duration of tests 24 months except Ref. 36,25 months; Ref, 37,27 months.

[b] Half male, half female except Ref. 37,75 males, 88 females.

[c] From 1960 and 1965 FDA stock.

TABLE 8

SURVIVAL RATES AND TUMOR INCIDENCES FOR OSBORNE-MENDEL RATS FED ALDRIN AND DIELDRIN-CALCULATED FROM DATA OF FITZHUGH ET AL. (5)

DIETARY LEVEL PPM	24 MONTH SURVIVAL RATE %	NO. OF RATS MICROSCOPICALLY SECTIONED	TUMOR INCIDENCE %
	Control		
0	50	17	17.6
	Aldrin		
0.5	50	19	53
2	50	19	37
10	42	22	36
50	25	18	28
100	17[a]	11	45
150	4[b]	9	11
	Dieldrin		
0.5	42	22	36
2	63	23	35
10	25	18	22
50	21[b]	20	20
100	13[a]	18	17
150	4[a]	11	0

[a] P = 0.01 for difference from controls.

[b] P = 0.05 for difference from controls.

1970 as in 1967 (Table 7). Similarly, if the control incidence in the Fitzhugh et al studies had been as high in 1952, as it proved to be for the same colony in later years, then it could have been concluded by these investigators that aldrin and dieldrin were anti-tumorigenic rather than tumorigenic.

The salient factors suggested by Wilson in determining the teratogenicity of drugs are outlined in Table 10 and include: the chemical and pharmacological properties, the level - duration, and material modulation of dosage, disposition within the conceptus and susceptibility of species and individual. Table 11 is a summary of teratogenesis its causes, suggested mechanisms, and manifestations.

Teratogenic effects of aldrin, dieldrin, and endrin in hamsters and mice have been reported by Ottolenghi et al (11). Single oral doses of aldrin, dieldrin, and endrin, (1/2 LD_{50}) in corn oil and administratered to pregnant golden hamsters on day 7, 8, or 9 of gestation caused a high incidence of fetal death, congenital anomalies and growth retardation. (Most frequent defects were cleft palate, open eye, and webbed foot.) Pregnant CD-1 mice given equivalent oral doses of each pesticide on day 9 of gestation, showed similar anomalies without concurrent increase in fetal mortality or growth impairment.

A crucial point concerning the evaluation of genetic risks by chemicals is the choice of test systems on which to base practical decisions and permit valid assessments. It has been further suggested by Bridges (12) and others that the evaluation of the potential hazard essentially involves 5 stages of decision making. These are: 1) Is the agent mutagenic ? 2) Is the agent likely to be mutagenic to man ? 3) What dose of mutagen is being received or

TABLE 9

TUMOR INCIDENCES IN OSBORNE-MENDEL RATS FED ALDRIN, DIELDRIN AND ENDRIN OVER A MAXIMUM PERIOD OF 31 MO CALCULATED FROM DATA OF DEICHMANN ET AL.(8)

	MALE				FEMALE				ALL			
RATS	0 ppm	20 ppm	30 ppm	50 ppm	0 ppm	20 ppm	30 ppm	50 ppm	0 ppm	20 ppm	30 ppm	50 ppm
Aldrin												
Histologically examined	75	45	46	45	88	47	44	31	163	92	90	76
% with benign tumors	7	5	2.2	5	44	34	46	26	32	20	23	13
% with malignant tumors	19	7	13	4	24	9	9	10	21	8	11	7
% total tumours	26	11	15	9	68	43	55	36	48	27	34	20
Dieldrin												
Histologically examined	75	48	38	44	88	48	41	41	163	96	79	85
% with benign tumors	7	2.1	5	2.3	44	38	27	29	32	20	16	15
% with malignant tumors	19	6	13	0	24	10	12	10	21	8	13	5
% total tumors	26	8	19	2.3	68	48	39	39	48	28	29	20
Endrin												
	0 ppm	2 ppm	6 ppm	12 ppm	0 ppm	2 ppm	6 ppm	12 ppm	0 ppm	2 ppm	6 ppm	12 ppm
Histologically examined	75	47	44	42	88	48	45	49	163	95	89	91
% with benign tumors	7	9	5	0	44	55	47	33	32	33	26	18
% with malignant tumors	19	15	9	24	24	21	11	22	21	18	10	23
% total tumors	26	24	14	24	68	76	58	55	48	50	36	41

TABLE 10

FACTORS DETERMINING TERATOGENICITY OF DRUGS

1. Type of Drug (Chemical and Pharmacological Properties).
2. Level and Duration of Dosage.
3. Maternal Modulation of Dosage
4. Access to the Conceptus.
5. Developmental Stage at Time of Dosage.
6. Disposition Within the Conceptus.
7. Susceptibility of Species and Individual.

TABLE 11

SUMMARY OF TERATOGENESIS

CAUSES → MECHANISMS → MANIFESTATIONS

CAUSES

Action of an agent from the environment on the embryo or the germ cells,

e.g. Radiations
- Chemicals
- Dietary deficiency
- Infection
- Hypoxia, etc.
- Temperature
- Endocrine imbalance
- Physical trauma
- Placental failure

MECHANISMS

Reaction within the embryo or germ cells, such as one or more of following:
- Mutation
- Chromosomal nondisjunction
- Mitotic interference
- Altered nucleic acid integrity or function
- Lack of precursors, substrates, etc.
- Altered energy sources
- Changed membrane characteristics
- Water-electrolyte imbalance
- Enzyme inhibition

MANIFESTATIONS

Pathogenesis, initiated by one or more of followin
- Cell death
- Mitotic rate change
- Reduced biosynthesis
- Altered differentiation schedules
- Impeded morphogenetic movement
- Etc.

- and leading to abnorm tissue and organ develo ment which determine th

NATURE AND INCIDENCE of FINAL DEFECT

TABLE 12

MAMMALIAN TEST SYSTEMS

TEST SYSTEM	ADVANTAGES	DISADVANTAGES	PRESENT STATUS	FUTURE DEVELOPMENT
Host-mediated assay	Only feasible screening method that detects point mutation, can detect transient metabolities as well as directly acting chemical. Indirect detoxification as well as potentiation by comparison with direct test on indicators. Simple, economical test. Moderately skilled investigator required. Used to correlate carcinogenicity with mutagenicity.	Indirect indication of active metabolite as to organ or tissue may be difficult.	Practical screening procedure over 100 compounds	Indicators, such as mammalian cells, that can divide in host and mutate at same rate as in vitro (i.e. mouse system). Localization of genetic effect in various organs. In bacteria, detection of multiple genetic events with a single indicator.
Cytogenetic, direct analysis (mitotic)	Applicable to a variety of hosts. Direct observation of chromosomal abnormalities. Somatic and germ cells can be analyzed comparatively low in cost. Moderately time consuming. Can be adapted to standard toxicological protocol.	Needs highly trained investigator. Quantitative correlation to point nutations not known. Usually detects a high percentage of nonviable cells. Chromatid aberrations mainly detected.	Practical screening procedure	Anaphase determination. Micronuclei determination. Application of newer straining techniques. Automation of chromosome analysis.

TABLE 12 (CONTINUED)
MAMMALIAN TEST SYSTEMS

TEST SYSTEM	ADVANTAGES	DISADVANTAGES	PRESENT STATUS	FUTURE DEVELOPMENT
Dominant lethal	Genetic test conducted in variety of hosts. Easy to carry out by trained technician. Fits into ongoing toxicity protocol. Moderate cost and time. Determines stages of spermatogenesis affected. Can be carried out on either chronic, sub-acute, or acute basis.	Test for lethality. Indirect test for cytogenetic abnormalities. Sensitivity presumably less than viable inherited abnormalities.	Practical screening procedure.	Cytogenetic analysis of live embryos. Development of improved statistical procedures. A higher precision in timing of affected stages of spermatogenesis.

is likely to be received by the population or individual's at risk ? 4) What is the risk from being exposed to the agent and 5) What is the acceptable risk ? Analogous considerations could also apply for carcinogens and teratogens.

A number of procedures are presently available in mammals, the majority of relatively recent origin, that can be used to determine the mutagenic activity of chemicals. Table 12 lists what is generally considered the three most useful test systems (e.g., host-mediated assay, dominant lethal and *in vivo* cytogenetic) in terms of their advantages, disadvantages, present status, and future development. It is well recognized that other test systems are also available or are being developed and refined, e.g., ranging from bacterial, fungal, plant, insect, mammalian cell culture, and intact mammal to reveal genetic damage, such as dominant lethality, translocations, deletions and duplications and non-disjunction, chromosome aberrations and forward and/or reverse, multiple specific locus, and induced recombination gene mutations. Tier approaches to the assessment of mutagenicity are also advocated (13) by investigators such as Flamm, De Serres and Bridges.

Table 13 lists the cytogenetic andmutagenic effects of DDT that have been reported, and once again illustrates the conflicting results and the difficulty of assessment of the mutagenic status of this most important insecticide.

With the apparent diminishing use of the chlorinated hydrocarbon and cyclodiene insecticides (primarily DDT and dieldrin) in the U.S. and many European countries, the organophosphorus and carbamate insecticides are assuming an increasingly important role. What are the possible consequences of the enhanced use of these replacement

TABLE 13

CYTOGENIC AND MUTAGENIC EFFECTS OF DDT

SPECIES OR SYSTEM	EFFECT	REFERENCES
Mouse	Chromosomal Damage	Johnson, G. A., and Jalal, S. M., J. Heredity, 63 (1973) 7
Mouse	Chromosomal Damage	Markaryan, D. W., Genetika, 2 (1966) 132
Rat	Non-Cytogenetic	Legator, M. S., Palmer, K. A., and Adler, I. D., Toxicol. Appl. Pharmacol., 24 (1973) 337
Rat Kangaroo Cell Line in Culture	Chromosomal Damage	Palmer, K. A., Green, S., and Legator, M. S., Toxicol. Appl. Pharmacol., 22 (1972) 355
Chinese Hamster Cell Line	DDT: Non-Cytogenetic, Non-Mutagenic DDE: Cytogenetic	Kelly-Garvert, F., and Legator, M. S., Mutag. Res., 17 (1973) 223
Drosophila Melanogaster	Weakly Mutagenic	Vogel, E., Mutat. Res., 16 (1972) 157
Mouse (Dominant-Lethal Test)	Mutagenic	Epstein, S. S., et al, Toxicol. Appl. Pharmacol. 23 (1972) 288
Rat (Dominant-Lethal Test)	Marginally Mutagenic	Palmer, K. A., Green, S., and Legator, M. S., Food Cosmet. Toxicol., 11 (1973) 53
Host-Mediated Assay (S. Typhinurium)	Weakly Mutagenic	Buselmaier, W., et al., Biol. Zentral. Bl., 91 (1972) 311
Wasp (Bracon Hebetor), Brime Shrimp	Non-Mutagenic	Grosch, D. S., J. Econ. Entomol., 60 (1967) 1177, Grosch, D. S., Science, 155 (1967) 592

TABLE 14

ORGANOPHOSPHORUS INSECTICIDES

AGENT	SPECIES OR SYSTEM	EFFECTS	REFERENCE
Malathion	Chick Embryo in Ovo	Teratogenic	Walker, N. E., Toxicol. Appl. Pharmacol., 19 (1971) 590
	Wistar Rat	Teratogenic	Dobbins, P. K., J. Fla. Med. Assn., 54 (1967) 452
	Human	Chromosome	Czeizel, A., et al, European Env. Mutagen Soc. Meeting, UPPSALA, Sweden, June 4-7 (1973)
Parathion	Chick Embryo in Ovo	Teratogenic	Khera, K. S., Toxicol. Appl. Pharmacol., 8 (1966) 345
	Rat	Teratogenic	Fish, S. A., Am. J. Obstet. Gynecol., 96 (1966) 1148 Kimbrough, R. D., and Gaines, T. B., Arch. Env. Hlth., 16 (1968) 805
	Human	Chromosome Anomalies	Czeizel, A., et al, European Env. Mutagen Soc. Meeting, UPPSALA, Sweden, June 4-7 (1973)
	Rat, Guinea Pig	Cytogenic Changes	Dikshith, T. S. S. and Datta, K. K., Bull. Env. Contam. Toxicol., 9 (1973) 65; Dikshith, T. S. S., Env. Physiol. Biochem., 3 (1973) 161

TABLE 15

ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

AGENT	SPECIES OR SYSTEM	EFFECTS	REFERENCES
Methyl Parathion	Rat, Mouse	Embryotoxic	Tanimura, T., et al., Arch. Env. Hlth., 15 (1967) 613 Fish, S. A., Am. J. Obstet. Gynecol., 96 (1966) 1148
	Human	Malformations (?)	Ogi, G., and Hamada, A., J. Jap. Obstet. Gynecol. Soc., 17 (1965) 569
Diazinon	Chick Embryo in Ovo	Teratogenic	Marliac, J. P., Toxicol. Appl. Pharmacol., 7 (1965) 490
	Rat	Teratogenic	Dobbins, P. K., J. Fla. Med. Assn., 54 (1967) 452
	Human	Chromosome Anomalies	Czeizel, A., et al, European Env. Mutagen. Soc. Meeting, UPPSALA, Sweden, June 4-7 (1973)
Carbaryl (Sevin)	Guinea Pig, Dog	Teratogenic	Robens, J. F., Toxicol. Appl. Pharmacol., 15 (1969) 152 Smalley, H. E., et al, Toxicol. Appl. Pharmacol., 13 (1968) 392
	Hamster, Rabbit, Mouse	Non-Teratogenic	Weil, C. S., et al, Toxicol. Appl. Pharmacol., 21 (1972) 390
	Rat, Mouse	Carcinogenic	Andrianova, M. M. and Alekseev, I. V., Vop. Pitan, 29 (1970) 71 Zabezhinsky, M. A., Vop. Onkol., 16 (1970) 106
	Rat	Teratogenic	Schtenberg, A. I. and Ozhovan, M. V., Vop. Pitan., 30 (1971) 151
	Rat	Non-Teratogenic Non-Mutagenic	Weil, C. S., et al, Toxicol. Appl. Pharmacol., 26 (1973) 621
	Guinea Pig	Non-Teratogenic	Weil, C. S., et al, Toxicol. Appl. Pharmacol., 26 (1973) 621
	Drosophila Melanogaster	Mutagenic	Brzheskiy, V. V., Genetika, 8 (1972) 151

insecticides ? The organophosphorous compounds such as malathion, parathion, and methyl parathion currently in use, as well as the carbamate insecticide Carbaryl (Sevin) are generally considered non-persistent and have a margin of safety between their effective use concentrations and their minimum toxic concentrations to mammals. They are however not without their potential carcinogenic, mutagenic, and teratogenic risk as shown in Tables 14 and 15.

In summary, some of the more salient characteristics of teratogenicity, mutagenicity and carcinogenicity (particularly the latter) have been examined and a number of conflicting, contradictory, and ambiguous examples in regard to the above toxicities of several organo chlorine, organo phosphorus, and carbamate insecticides have been cited.

It is suggested that a major step could be taken to ameliorate the possibility of ambiguity in toxicity testing if there were a central repository of well characterized environmental, carcinogen, mutagen, and teratogen chemical standards. Perhaps the WHO might well serve as a focal point in this capacity.

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DISCUSSION

EPSTEIN (U.S.A.)

Suggested distinctions between tumorigens and carcinogens, have been repeatedly examined by a wide range of expert national and international committees and have been shown to be totally lacking in validity. Illustratively, it has been claimed that DDT and Dieldrin should be recorded as tumorigenic because both produce mouse hepatomas which are claimed to be "benign tumors". However, DDT induced hepatomas metastisizing to the lungs if mice are not prematurely sacrificed, DDT also produces malignant lymphomas in mice. Dieldrin has been shown in a wide range of studies including those recently published by SHELL Toxicologists (Thorpe & Walker, 1972/1973) to produce hepatomas in mice which metastasize to the lungs, and a wide range of extra-pulmonary malignant tumors. Dieldrin also provides liver cancers and malignants in other organs in rats (Thorpe & Walker, 1973). These hepatic and non-hepatic neoscosms in mice and rats following dieldrin treatment occurred in statistically significant incidences. The unequivocal carcinogenicity of aldrin and dieldrin has been recently summarized in testimony, at recent (1974) hearings in litigation between EPA and EDF vs SHELL, by Saffiotti, U., Farber, E., and Epstein, S.S.

FISHBEIN (U.S.A.)

The comments of Dr. Epstein on the carcinogenicity of DDT and dieldrin are noted and appreciated. The "unequivocal carcinogenicity of aldrin and dieldrin" which was referred to by Dr. Epstein was derived from calculations performed on the data (on the incidence of liver-tumors of CF-1 mice) of Walker, Thorpe, and Stevenson (Food Cosmet. Toxicol., 11, 415-432, 1972) employing calculations as described by Mantel and Bryan (J. Nat. Cancer Inst., 27, 455-470, 1969) using an assumed slope on one probit per log dose, to calculate the dose corresponding to a maximum risk of one per hundred million mice. The highest calculated dose corresponding to a risk of 10^{-6} was obtained with the data at 1.25 parts per million of dieldrin in the diet fed CF-1 mice for 128 weeks. The values for Pmax for the experimental points and Pmin for the controls were based on two-tailed 95% confidence limit binomial tables, and hence correspond to 97.5% one-tailed confidence limits.

With regard to the comment of Dr. Epstein that dieldrin has been shown to produce hepatomas in mice which metastasize to the lungs, this was observed in the study of Walker et al (Food Cosmet. Toxicol., 11, 415-432, 1972) in a male and a female mouse (out of 60 animals) fed 2.5 ppm dieldrin for 128 weeks. The lung lesions comprised massive secondary tumors and a small emboli of tumor cells in pulmonary vessels.

It is also important to note that a new criterion for determining the carcinogenicity of a substance was used in the Environmental Protection Agency (U.S.) decision of October 1,1974 t suspend all major uses of aldrin and dieldrin pesticides. For purposes of "carcinogenicity testing", tumorigenic substances and carcinogenic substances are "synnymous" and a carcinogenic substances is defined as "one that increases the incidence of benign or malignant tumors in exposed animals, decreases the latency period between exposure and onset of tumor, or results in unusual tumors" (Chem. Eng. News 52, 13, 1974; Federal Register, Oct. 18, 1974, p. 37246).

OLOFFS (Canada)

In view of the last comments, it should also be mentioned that O_1p' - DDD, administered orally to humans at doses as high as 10 gr/day for several months, has been used in the past for the treatment of adrenocortical cancer.

TOXICOLOGY OF ATMOSPHERIC POLLUTANTS RESULTING FROM FUEL ADDITIVES AND EMISSIONS ASSOCIATED WITH THE USE OF AUTOMOBILE CATALYTIC CONVERTERS

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Environmental Protection Agency, National Environmental Research
Center, Environmental Toxicology Research Laboratory, Cincinnati
Ohio, USA

ABSTRACT

The purpose of this paper is to present the definitive toxicologic investigative approach employed in our laboratory for testing automotive emissions, fuel additives, and emission components related to the catalytic converter emission control system.

A matrix for investigating the toxicology of individual pollutants and emissions from mobile sources has been used to determine the potential toxic effects. The presented data are illustrations of the response of selected animal models to the catalyst-treated exhaust and to individual components such as platinum and palladium. The biological parameters studied were markedly influenced by the use of the converter.

Introduction

The Environmental Toxicology Research Laboratory, NERC-Cincinnati is engaged in the testing, evaluating and defining potential harmful effects of environmental pollutants from mobile and stationary sources. The data obtained in experimental biological models provide necessary input for the development of scientific criteria, which in turn, serve in the process to establish realistic environmental standards. During the past year the toxicological investigations conducted in this laboratory dealt primarily with studies of individual catalytic converter emission components, and exposures of animals to whole emissions from engines equipped with or without catalysts. Emphasis was placed in this area because the United States automotive manufacturers have developed and will install on many of the 1975 light-duty vehicles catalytic converters to reduce concentrations of carbon monoxide and hydrocarbons in exhaust emissions.

Since human exposure from this source is particularly related to air pollution hazards, the route of animal exposure is primarily by inhalation. In many instances, however, the pollutants e.g. heavy metal compounds, are found in more than one environmental media and to provide relevant information on their public health impact, several routes of entry are investigated. In addition, appropriate mammalian species of different ages (from embryonic to aged models) are being used to optimize the possibility of reproducing the human response in the different segments of the population. The experimental protocol for the definitive toxicologic investigations is based on a matrix which is summarized in Table 1. It describes three types of investigations conducted in this laboratory.

Table 1

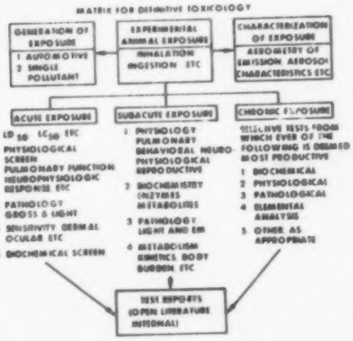

MATRIX FOR DEFINITIVE TOXICOLOGY
GENERATION OF EXPOSURE
1 AUTOMOTIVE
2 SINGLE POLLUTANT
EXPERIMENTAL ANIMAL EXPOSURE
INHALATION INGESTION ETC
CHARACTERIZATION OF EXPOSURE
AEROMETRY OF EMISSION AEROSOL CHARACTERISTICS ETC
ACUTE EXPOSURE
1 LD50 LC50 ETC
2 PHYSIOLOGICAL SCREEN PULMONARY FUNCTION NEUROPHYSIOLOGIC RESPONSE ETC
3 PATHOLOGY GROSS & LIGHT
4 SENSITIVITY DERMAL OCULAR ETC
5 BIOCHEMICAL SCREEN
SUBACUTE EXPOSURE
1 PHYSIOLOGY PULMONARY BEHAVIORAL NEURO-PHYSIOLOGICAL REPRODUCTIVE
2 BIOCHEMISTRY ENZYMES METABOLITES
3 PATHOLOGY LIGHT AND EM
4 METABOLISM KINETICS BODY BURDEN ETC
CHRONIC EXPOSURE
SELECTIVE TESTS FROM WHICH EVER OF THE FOLLOWING IS DEEMED MOST PRODUCTIVE
1 BIOCHEMICAL
2 PHYSIOLOGICAL
3 PATHOLOGICAL
4 ELEMENTAL ANALYSIS
5 OTHER AS APPROPRIATE
TEST REPORTS (OPEN LITERATURE INTERNAL)

Fig. 1. A closed "nose only" rodent inhalation chamber

(1) The short-term acute exposure experiments in which high levels of new compounds are tested to ascertain frank systemic effect;(1) (2) Subacute exposure experiments of longer duration at lower levels where specific bioeffect end-points are measured to provide physiological, biochemical and pathological parameters of injury; and (3) Chronic, long-term, exposure studies conducted at realistic environmental levels.(2,3) The approach for any given pollutant will vary somewhat from the general scheme depending on what are the gaps in current knowledge concerning the compound under investigation.

A. Animal Exposure Systems

For the single pollutant inhalation exposure studies, several aerosol and gas generating systems are presently in use. An example of a closed, "nose only" inhalation animal chamber system is shown in Fig. 1. The inhalation chamber permits exposure of 80 rodents simultaneously to high level aerosols or gases including radioactive metal tracers. In addition to this system, the inhalation capability of the laboratory includes 24 large animal chambers (approx. 3 m^3 ea.) and 25 smaller portable chambers (approx. 1/2 m^3 ea.), and among others, a single animal aerosol generation system to measure pulmonary mechanics and cardiovascular parameters in animals during exposure. Another development during the past year includes the use of telemetry for measurements of respiratory and cardiovascular parameters in large animals (dogs or primates) which are being chronically exposed to various pollutants in the larger chambers.(4)

B. Automotive Engine and Exhaust Exposure Systems

The installation of two engines (Ford 1975 prototype engine,

Table 2

15 MPH STEADY SPEED RUNS (TENTATIVE)
(Exhaust Emissions* Normalized to 7.5/1 Dilution)

Gaseous:	Indolene (0.05% S) With Converter (C)	Indolene (0.10% S) With (C)	Indolene (0.10% S) Without (C)
CO, ppm	7	8	522
THC, ppm	9	9	99
Particulate:			
Total, mg/m	9.6	30.2	3.2
Sulfate, mg/m	4.8	12.8	0.5
Nitrate, mg/m	.01	.01	.04

*Measured in N-1 exposure chambers

Table 3

AVERAGE PERCENT CONCENTRATION
REDUCTIONS OF TAME J OVER I
(W/O CATALYST)

CO	93%
THC	80%
ALDEHYDES AND ACETYLENE	99+%
OLEFINS	93%
ALIPHATICS	59%

Fig. 2. G.M. engine with "pelletized" catalytic converter

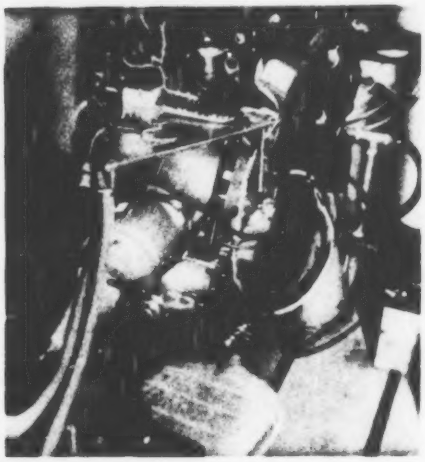


Fig. 3. 1975 Ford prototype engine with monolith catalytic converter

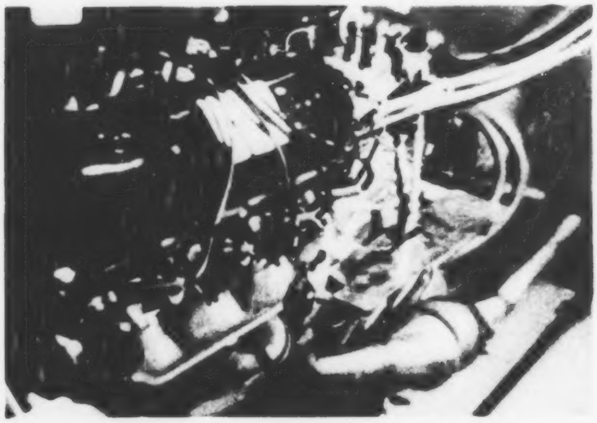


Fig. 4. Dilution chamber for studies of exhaust emissions

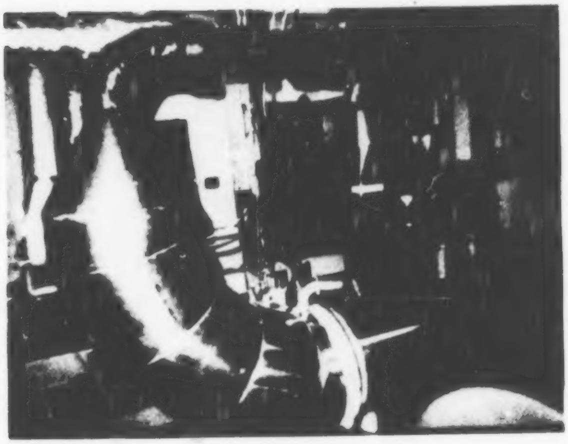


Fig. 5. Irradiation chamber

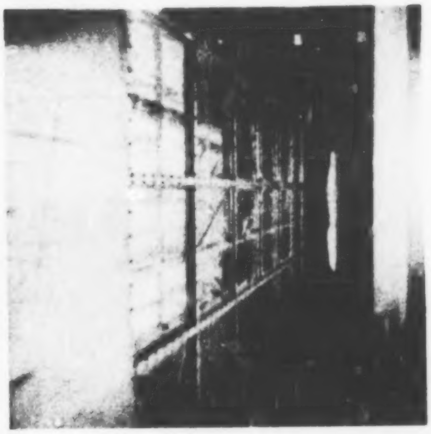


Fig. 6. Animal exposure chambers for exhaust emission studies

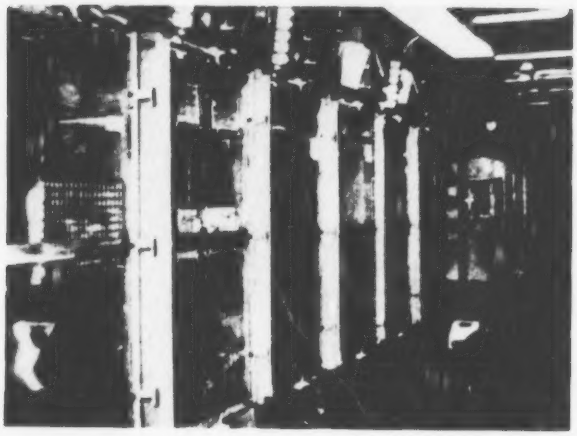
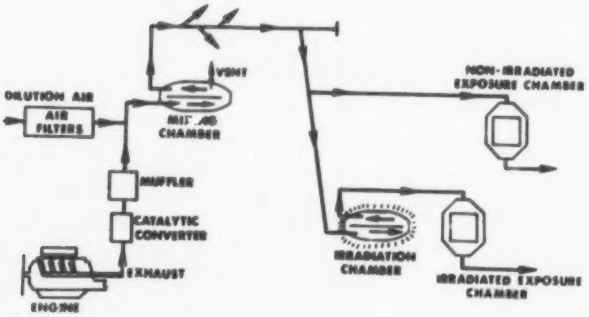


Fig. 7. Schematic diagram for toxicologic testing of automotive emissions



Chevrolet 1973 production engine with control devices) equipped with catalytic converters enabled the laboratory to conduct subacute and chronic inhalation studies of the emissions with and without the catalyst.(5) Simultaneously, aerometry measurements were made of the emission components as a part of any of the Toxicologic Assessment of Mobile Emissions (TAME) experiments. Figures 2-6 provide a pictorial view of the exposure system. They include the G.M. and the Ford engines with the catalytic converters, a dilution pipe, irradiation chambers for production of photochemical smog, and 28 animal exposure chambers connected to this system (approx. 1.25 m^3 ea.). The schematic diagram of the system, as well as aerometry sampling ports is presented in Figure 7.

C. Aerometry of the Catalyst Emissions

From the standpoint of potential health effects, the emission components of greatest concern following the use of the catalyst is the increased amount of particulates, particularly sulfates,(6) and the potential release of catalytic attrition products into the atmosphere containing the noble metals platinum and palladium.(7) In addition, a shift in the individual hydrocarbons is also of potential interest. Table 2 summarizes the effects of the catalyst control system in the 1973 G.M. engine on the exhaust emissions. The data show that the oxidizing catalyst has successfully reduced the levels of carbon monoxide and total hydrocarbons in the exhaust stream; however, the amount of particulates was significantly increased, especially the amount of sulfates. A striking increase occurred in the acidity of the particulate. In one of our experiments with the catalyst in place, the acidity of particulates were measured to be some 65 times higher than in the exhaust without

Table 4

RELATIVE ACIDITY LEVELS OF
EXHAUST EMISSION PARTICULATE AT 24.2 km/Hr. (15 MPH)

Operating Condition –	No Catalytic Converter	With Catalytic Converter	
	High-S Indolene fuel	Reg Indolene	High-S Indolene
Relative Acidity of Particulate	1	65	260
Total Particulate @ 7.5/1 Dilution mg/m^3	2.7	7.2	25.6

Fig. 8. Mortality rate in infant rats

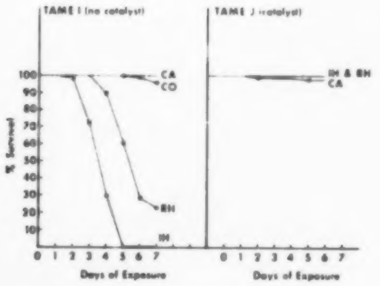


Fig. 9. Body weight of lactating female rats

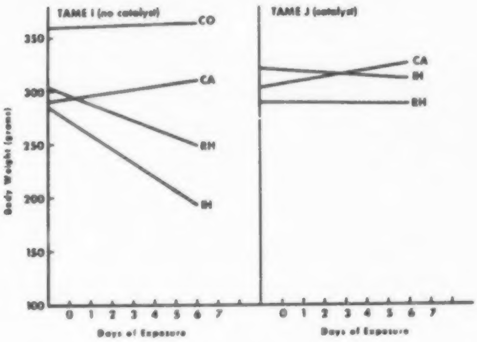


Table 5

SURVIVAL OF SUCKLING RATS FOLLOWING EXPOSURE TO WHOLE AUTOMOBILE EXHAUST

	TAME I	TAME J
CLEAN AIR CONTROL	100%	98%
NONIRRADIATED EXHAUST	23%	100%
IRRADIATED EXHAUST	0%	100%
CARBON MONOXIDE CONTROL	96%	

Clinical studies have documented a relationship between lead intoxication and behavioral disorders in children (Byers and Lord [1]; Chisolm and Harrison, [2]; De la Burdee and Choate [3]; David, et al, [4]). These disorders have been described as irritability, restlessness and agressiveness. Studies in adult animal models have not reproduced these findings which supports clinical knowledge on the relationship between age and susceptibility to lead. Recently, it has been demonstrated that animals exposed to inorganic lead during critical periods of neurological development (analogous to early childhood exposure via pica) develop the behavior disorder of hyperactivity (Silbergeld and Goldberg, [5,6,7]; Sauerhoff and Michaelson, [8]; Allen, et al, [9]). Hyperactivity is seen in animals exposed to levels of lead below that which produces severe growth retardation, kidney damage, paraplegia, or convulsions.

THE ANIMAL MODEL - The intention of this animal model is to provide an analogue to childhood lead poisoning in terms of equivalent age, gastrointestinal route and chronic nature of low level lead exposure. Inorganic lead (2,5, or 10 mg/ml lead acetate) was administered to the mother in the drinking water and through her to the nursing offspring which were the subjects of study. These exposure levels of lead produced a dose-related reduction of growth (about 10% at 5 mg/ml) and a retardation of several developmental landmarks (Table 1). The offspring have no signs of acute lead poisoning but in analyses conducted in collaboration with Dr. J.J. Chisolm. alterations in heme synthesis were found associated with a blood lead level of 50-80 μg%.

TABLE 1. DEVELOPMENTAL LANDMARKS IN CONTROL AND LEAD-TREATED MICE[a]

	Day of Occurrence	
Landmark	Controls	Lead
Eye Opening	13	17
Full Incidence of Hair	16	20
Coordinated Walking	18	25
Weaning	22	30

[a] Data from Silbergeld and Goldberg, [5].

Between 30 and 150 days of age, these animals were individually tested for levels of spontaneous motor activity. In all cases, the lead-treated animals were about 3 times as active as coetaneous controls (Figure 1).

A number of points should be made about this animal model. Increases in the level of lead exposure do not increase hyperactivity but induce other sequelae of lead poisoning. Animals whose lead exposure terminates at weaning also evince hyperactivity as adults. However, animals exposed to lead only post-weaning do not develop the behavioral dysfunction, suggesting that the behavioral toxicity of lead is dependent upon the accessability and immaturity of the developing nervous system.

exhaust exposed hamsters, the pulmonary changes were not as severe. Pulmonary changes noted in the rats exposed to non-catalytic treated exhaust paralleled those found in the hamsters. In both the irradiated and nonirradiated exhaust exposed hamsters (TAME I), some vacuolar changes were found in hepatic parenchymal cells after 5 days of exposure. Similar vacuolar changes in the renal tubular cells were seen in the irradiated exhaust exposed hamsters. In the catalyst-treated exhaust exposures, TAME J, no significant treatment-related morphologic changes were noted in either the rat or hamster tissues. In the clinical pathologic tests performed on animals exposed to the catalytic-treated exhaust (TAME J), the only statistically significant treatment effect was an increase in total serum proteins. In the animals exposed to the noncatalytic treated exhaust (TAME I), there were statistically significant treatment effects in both nonirradiated and irradiated exhaust exposures on total protein, platelet count, RBC and WBC counts, white cell differential, alkaline phosphatase, HB, HCT, PTT, SGOT, and SGPT levels. The irradiated exhaust also produced a treatment effect on levels of BUN and fibrinogen. It should be noted that the exhaust exposed animals in the noncatalytic-treated groups showed a rather striking increase in hemolysis resistant RBC's, which necessitated manual determinations of WBC counts. In explaining the effects, the RBC related changes and alkaline phosphatase levels could relate to the high levels of CO; the WBC changes most probably relate to the severe acute inflammatory response in the lungs; the other changes could relate to hepatic and/or renal dysfunction.

Fig. 12. Retention of ^{103}Pd and ^{103}Pt in rats

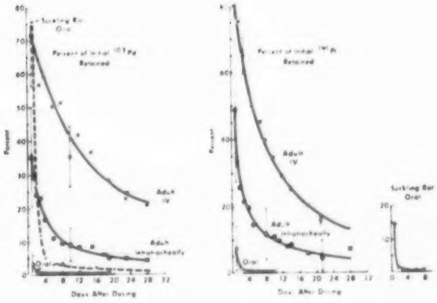


Fig. 13. Retention of $^{191}PtCl_4$ and $^{191}Pt(SO_4)_2$ in rats following inhalation

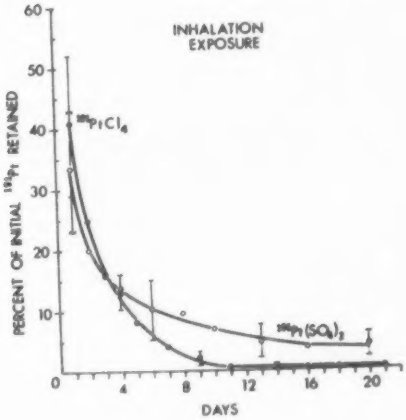


Table 2.

EFFECTS OF DRUGS ON LEAD-INDUCED HYPERACTIVITY [a)]

DRUG (Dose mg/kg)	EFFECT ON LEAD-TREATED HYPER-ACTIVE CONTROLS		COMMENTS
α-methylparatyrosine (50)	--[b)]	0	In treated, produces "normal" response to amphetamine; in controls, abolishes amphetamine response
d-amphetamine (10)	--	++	In controls, some stereotypy
dimethylaminoethanol (200)	--	+	
fenfluramine (20)	--	+	
methylphenidate (25, 40)	--	++	
oxotremorine (0.08)	-	0	
physostigmine (0.1-0.5)	--	0	
apomorphine (10)	0	-	In treated, induces stereotypic circling and increases reactivity
atropine (0.01)	0	0	In treated, increases reactivity
benztropine (15)	+	0	In treated, increases reactivity
l-dopa (50)	++	0	In treated, increases reactivity
phenobarbital (20)	++	0	

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a) Some of these data are reported in Silbergeld and Goldberg, (6,7). The rest is presented in material submitted for publication.

b) +=increase in activity; -=decrease in activité; O=no change. Number of symbols denotes qualitative amount of change; -- in treated=suppression of activity to that of predrug baseline of control animals.

It is not presently known if the other aminergic agonists methylphenidate and fenfluramine act similarly on the cholinergic system.

NEUROCHEMISTRY - The transport of neurochemicals into neurons has been proposed as an important step in the regulation and turnover of suspected neurotransmitters and may be a valuable method for studying CNS function (Kuhar, [10]). High affinity transport systems are observed experimentally by the low concentrations against which neural tissue will accumulate the compounds. Thus, changes in high affinity transport may reflect alterations in neurotransmitter function. Many of the drugs found to have differential effects on lead-induced hyperactivity are known to affect the high affinity transport of the catecholamines and acetylcholine. In 40-60 day old lead-induced hyperactive mice the high affinity transport of dopamine, choline and tyrosine were significantly affected (Table 3). The transport of other suspected neurotransmitters and a non-neuroactive compound, leucine, were not changed.

The steady state levels of acetylcholine, norepinephrine, and dopamine were also measured in hyperactive animals. Preliminary results indicate that only norepinephrine levels are changed (25% increase). This is consistent with the increases in the transport of tyrosine, a precursor of norepinephrine, and with the suppression of lead-induced hyperactivity by α-methylparatyrosine, a compound which inhibits the synthesis of norepinephrine (Table 2).

CONCLUSIONS - Low level lead exposure has been shown to interfere with central nervous system function and to result in retardation of development, in hyperactivity, and in alterations in response to many pharmacological agents. The mechanism of action is probably related to an inhibition of central

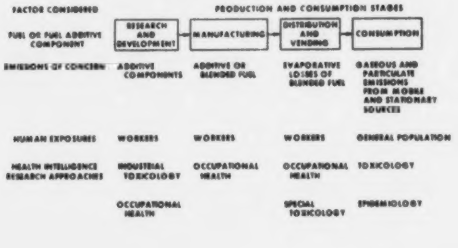
Table 8

VISUAL-EVOKED POTENTIAL SCREEN

METAL COMPOUNDS	REPRODUCIBLE DOSE-EFFECT THRESHOLD (mg/kg)
COBALT	0.010
CADMIUM	0.10
CHROMIUM	0.40
PALLADIUM	0.40
BARIUM	2.0
MANGANESE	2.0
PLATINUM	MINIMAL EFFECT

Table 9

Overview of Public Health Impact of Fuels and Fuel Additives



FACTOR CONSIDERED	PRODUCTION AND CONSUMPTION STAGES			
FUEL OR FUEL ADDITIVE COMPONENT	RESEARCH AND DEVELOPMENT	MANUFACTURING	DISTRIBUTION AND VENDING	CONSUMPTION
EMISSIONS OF CONCERN	ADDITIVE COMPONENTS	ADDITIVE OR BLENDED FUEL	EVAPORATIVE LOSSES OF BLENDED FUEL	GASEOUS AND PARTICULATE EMISSIONS FROM MOBILE AND STATIONARY SOURCES
HUMAN EXPOSURES	WORKERS	WORKERS	WORKERS	GENERAL POPULATION
HEALTH INTELLIGENCE RESEARCH APPROACHES	INDUSTRIAL TOXICOLOGY OCCUPATIONAL HEALTH	OCCUPATIONAL HEALTH	OCCUPATIONAL HEALTH SPECIAL TOXICOLOGY	TOXICOLOGY EPIDEMIOLOGY

provides the decision-making bodies with information on the potential toxicity of new substances or compounds and in this way fulfill a vital function. Table 9 summarizes the public health overview and the role of toxicology in manufacturing, distribution, and consumption of fuel additives. It lists the population subgroups which may be exposed to these compounds. It is in this manner that toxicologists can optimally coordinate their findings with the epidemiologists and clinicians.

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DISCUSSION

STUPFEL (France)

I have always been impressed by the beautiful and precise toxicological research of the Cincinnati Group which is a pionneer in the field of automotive exhaust biological effects. I take this opportunity to ask 2 questions to one specialist of this group:

1. In your gases exposure experiments do you leave the food in the chamber so it could be contaminated?

2. What is the time of your exposure (24 hours a day or less), For nocturnal rodents we have observed the importance of a 24 hours exposure especially during the dark - which is the active period - and where the threshold toxity of carbon monoxide (judged by the matabolic effects) is 50 ppm in the dark period and 600 ppm in the light period.

STARA (U.S.A.)

Thank you for your compliment, Dr. Stupfel.

As to your first question: We have conducted an 8 year study of air pollutant effects in beagle dogs - there the daily exposure period was 16 hrs/day in order to compare at least somewhat to usual heavier traffic condition in urban areas. In this experiment the dogs were fed during the 8 hours rest period. Presently we conduct exposure experiments in rodents, from one week to two months duration on a 24 hours exposure basis. We did have some problems with feed contamination and the animals not eating it; we now exchange feed more often and the animals are eating it as shown by weight gains similiarity between positive and negative (clean air) control groups.

I completely agree with your second comment dealing with the metabolic rate of rodents during light and dark cycle and the statement supporting 24 hours exposure of rodents. As a matter of fact we have performed experiments of effects of the light and dark cycle using mitotic rate index in the cornea of the eye of rodents and found significant differences.

SCHLIPKOTER (Federal Republic of Germanv)

1. To what extent did you dilute vehicle exhaust emissions in your excellent research inventigations?

2. How do you explain the high mortality rate in your acute studies, if this was not caused by the CO concentration in the exposure chambers?

STARA (U.S.A.)

1. For the present studies with the catalyst device emissions we dilute the auto exhaust at a ratio of 10 : 1 because we want to test the acute effects of auto emissions with and without the catalytic converters. However,we have the capability to lower the dilution down to realistic environmental levels, e.g. 200 : 1.

2. The high mortality rate in the weanling rats was caused by other emission components than the CO. The CO exposure group was our positive control group and resulted in a negligible mortality rate. The mortalitv was caused bv the combination of total hydrocarbons, nitrogen oxides and perhaps CO together in the raw exhaust (a total of 75% mortalitv rate), and additionalv the oxidants in the irradiated exhaust (a total of 100% mortalitv rate).

CALANDRA (U.S.A.)

What is your assessment of the significance to the general population of the safety of the catalyst and its attrition products as well as the increased emission of sulfates? This is a health issue and not an economic one.

STARA (U.S.A.)

My answer must be based in this forum on scientific data. Thus far, in our experiments of one week and one month continuous exposure, we have not found overt deleterious effects in rodents exposed to the exhaust from engines equipped with the noble metals oxidizing catalyst. That is not to say that longer chronic experiments may not show some positive results - perhaps. But so far there is no technical evidence available to prove this point either way.

Oh, you wish to know my personal opinion? Well, I would be happy to discuss the matter further with you, but not from this rostrum.

QUANTITATIVE ESTIMATION OF THE EFFECT OF VARIOUS PULMONARY IRRITANTS ON THE LUNG CELL POPULATION

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ABSTRACT

Polyvinylpyridine-N-oxide (PVNO) is a polymeric non-toxic substance which is utilised in the treatment of experimental and human silicosis.

After i-venous injection in Rats, PVNO accumulated in the macrophages of liver, spleen and bone marrow, but only poor in the lungs.

This distribution was reversed by the intra-tracheal injection of Phosphatydilserine, Gama-Globuline, Freund Adjuvant and the inhalation of NO_2*, and followed by a large accumulation of PVNO in the lung tissue. We suppose that this increase is caused by the migration to the lung of macrophages originating from the RES of the body and which have previously pynocitosed PVNO.*

Counts of the total number of cells recovered from the lung washings from rats which were treated with Freund Adjuvant, showed a good correlation between the progressive accumulation of PVNO in lung tissue and the progressive increase of the number of alveolar cells in the lung washings.

As part of an organ which comes first in contact with atmospheric pollutants and other pulmonary irritants, the alveolar macrophages play an important role in lung defence, which is accomplished by a certain number of alveolar macrophages. It is a still unresolved problem whether this defence is executed by an invariable amount of macrophages or if various pulmonary irritants can increase or diminish the number of alveolar macrophages in the lungs. The physiological and pathological mechanisms which elicit the increase or diminution of the alveolar macrophages are incompletelly investigated, tool. We suppose that because the lung macrophages are part of the general RES, any augmentation of lung macrophages suppose that supply is a secondary one inasmuch as the cells are furnished to the lung from the RE organs as a response to pulmonary injury.

The number of alveolar macrophages on the one hand probably depends from the chemical nature of the pulmonary irritant, its retention in the lung and the duration of the exposure, on the other hand from the age of the exposed being; all these relationships are in detail. In order to clarify this problem we have developed 2 methods to test the influence of various pulmonary irritants on the cell population of the lung.

The first method

Young and old rats were exposed to gaseous noxious agents that contaminate urban atmospheres, 24 h per day. At different times the animals were killed and the lungs were washed with saline. The alveolar macrophages were obtained from 23 lung washings per animal, using 1 ml of saline for each washing. The number of cells and the cell size was estimated with a Culter Counter, Model FN.

The second method

Rats were injected i-v which Polyvinylpyridine-N-oxide (PVNO) a polymeric non toxic substance which accumulate in the RES. By means of a quantitative method (GRÜNSPAN, 1, 2), we have estimated the amount of PVNO stored in the liver, spleen, bone marrow, and lungs 1-2 months after the i.v. application of PVNO, before and after the i-tracheal administrative of various substances or the inhalation of NO_2.

Following experimental groups are formed:

Group I - only PVNO i.v. (50 mg/kg body).
Group II - PVNO and 3 mg of Phosphatidylserin injected i-tracheally.
Group III - PVNO and 3 mg of Gama-Globuline injected i-tracheally.
Group IV - PVNO and 0,05 ml of Freund Adjuvant complet injected i-tracheally.
Group V - PVNO and 0,005 ml of Freund Adjuvant complet injected i-tracheally.followed by exposure to NO_2 (20 ppm) for 48 hr.
Group VI - PVNO followed by exposure to NO_2 for 7 days.
Group VII - PVNO followed by i-tracheally injection of 0,5 ml NaCl 0,9%.

In the experiments with Freund Adjuvant we have made also a lung lavage technique and the number and size of cells in the lavage liquid was counted and correlated with the amount of PVNO found in the lung.

RESULTS

1. Physiologic distribution of PVNO (50 mg/kg) in the normal Rat:
   When 50 mg PVNO/kg were injected i.v. to normal Rats 30 days after the injection, the liver contained 500 μg, the spleen 180 μg, the bone marrow 150 μg and the lungs only 50 μg of PVNO (fig. 1).
2. The accumulation of PVNO in the lung following Phosphatidylserine is important, and was estimated at 140 μg (fig. 2).
3. The i-tracheal injection of Gama-Globuline caused an accumulation of 100 μg in the lung (fig. 3).
4. The greatest accumulation of PVNO in the lung occured 7 days after the i-tracheal injection of Freund Adjuvant (200 ug) (fig. 4).

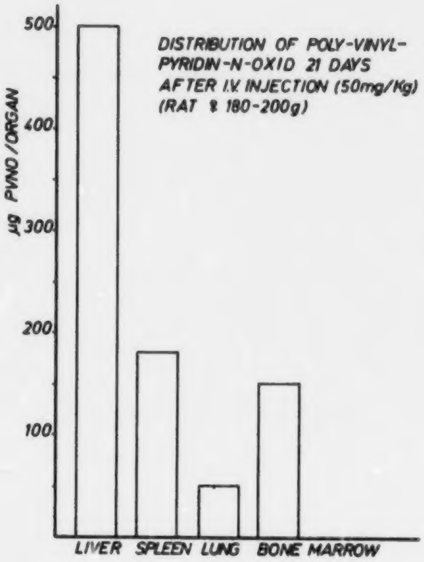

Fig. 1. Physiologic disposition of PVNO

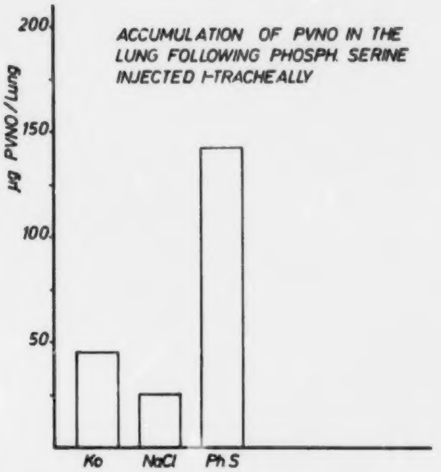

Fig. 2. Accumulation of PVNO in the lungs following i-tracheal injected Phosphatidylserine

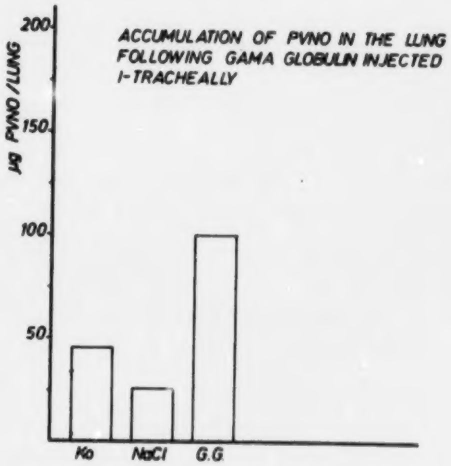

Fig. 3. Accumulation of PVNO in the lungs following i-tracheal injected Gama-Globuline

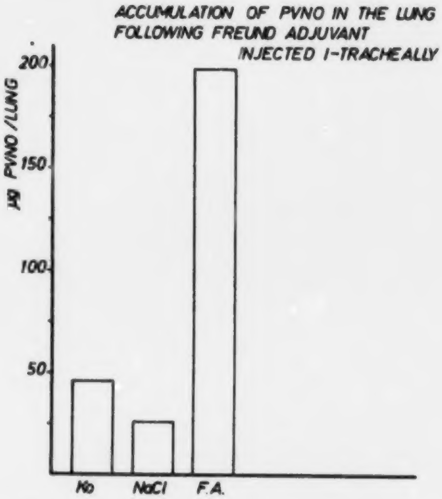

Fig. 4. Accumulation of PVNO in the lungs following i-tracheal injected Freund Adjuvant

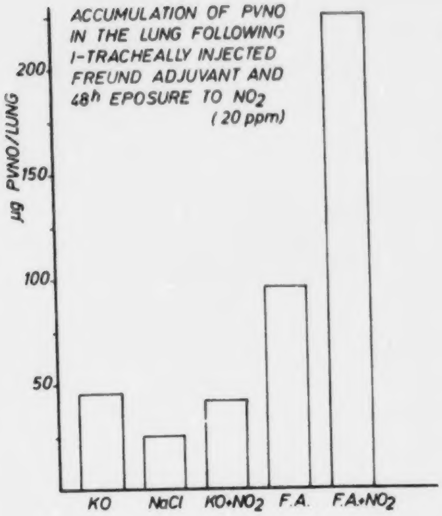

Fig. 5. Accumulation of PVNO in the lungs after Freund Adjuvant injected i-tracheally followed by exposure to NO_2 (20 ppm) for 48 hr.

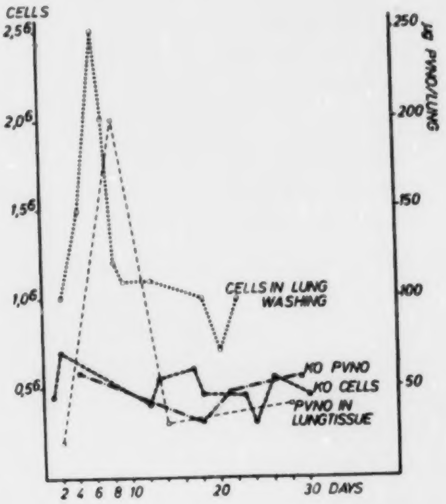

Fig. 6. Number of cells in lung washings and PVNO in lung tissue after i-tracheal injection of Freund Adjuvant

KO = Controls

NaCl = NaCl 0,9%

GG = Gamma Globuline

P.S. = Phosphatidylserine

F.A. = Freund Adjuvant

5. The exposure to NO_2 determine an accumulation of PVNO which is simular to those observed in the normal (fig.5).
6. In the Rats which have received i-tracheally Freund Adjuvant, and consecutivelly NO_2 for 48 hr., we have observed a large increase in the amount of PVNO in the lung tissues, which was greater that the increase caused by Freund adjuvant alone (fig. 5).

   Counts of the total number of cells recovered from the lung washings from Rats injected i-tracheally with Freund Adjuvants show a good correlation between the progressive accumulation of PVNO in lung tissue and the progressive increase of the number of alveolar cells in the lung washings (fig. 6).

The i-tracheally injection of NaCl not determine any accumulation of PVNO in the lung tissue (fig. 2, 3, 4).

DISCUSSION

The results of this experiments demonstrated that it is possible to charge the RES of the Rat by means of Polyvinylpyridine-N-oxide wich is pynocitosed in large amounts by the macrophages from the liver, spleen, bone marrow and only poor from the lung. By means of an i-tracheally injection of Phosphatidylserine, Gama-Globuline, or Freund Adjuvant alone or followed by NO_2, we can discharge the RES which mobilise the macrophages loaded with PVNO to the lungs.

If the amount of PVNO found in the lungs of the normal Rat 30 days after the i.v.-injection is only 40-50 µg, we assume that this counted exprime a proportionally number of macrophages which have pynocitosed PVNO and which are present in the lungs. We can accept that the amount of PVNO found in the lungs is a mesure of the number of RE cells accumulated in the lung as a direct consequence of an i-tracheally irritation.

This irritation started from the lungs, reaches the fixed cells of the RES which have previously pynocitosed the i.v.-injected PVNO and determine this cells to migrate to the lung. Our experiments demonstrate the existence of a mechanism which suppose an intimate cooperation between lungs and the RES of the body.

The fact that a component of the alveoles surfactant (Phosphatidylserine) and a substance involved in the host defense of the lung (Gama-Globuline) are able to determine the migration of the fix cells of the RES is currently under investigation.

Thus, the augmentation or diminution of the number of alveolar macrophages following exposure to various noxious agents can be quantified directly with the help of the Culter Counter or by measuring the PVNO content of Rat lung after labelling the cells with this nontoxic polymeric substance these estimations providing important insight into the defence of the lung because the alveolar macrophage ist inyolved both into the phagocytosis of inhaled particles and into the production of antibodies.

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LOCAL EFFECTS OF INHALED LEAD COMPOUNDS ON THE LUNG

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ABSTRACT

The effects of inhaled lead particles on the lung parenchyma are reported. The following findings were obtained:

1. *The ultrastructure of the alveolar macrophages and the pneumocytes type I indicate a cytotoxic action of the inhaled lead aerosol.*

2. *The pneumocytes type I exhibit an increased H 3-thymidine labelling index. This finding can be explained by elevated turn-over of cells at the alveolar wall.*

3. *The determination of benzopyrene hydroxylase activity showed a clear depression to 10% of the control values.*

4. *In a chronic 15 weeks experiment with alternate inhalation of lead and titan dioxide aerosols, lung clearance was impaired by 33 to 39%.*

The lead concentrations used were in the range of 70 to 170 $\mu g/m^3$*, i.e. close to the West German MAK-value.*

On the basis of these investigations it must be assumed that inhalation of lead compounds of the described or even lower concentrations produce local toxic effects in the lung.

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Introduction

Number and vitality of alveolar macrophages play an essential role in the defense mechanism of the lung against live and inanimate noxious agents. After BECK and collaborators (1) could demonstrate that lead compounds exhibit a considerable cytotoxicity in vitro comparative to that of quartz, we investigated the local effects of inhaled lead particles on the pulmonary alveolar macrophages and the alveolar parenchyma.

For this purpose four different test methods were employed:
1) electron microscopy; 2) determination of the H-3-thymidine index; 3) studies of the benzopyrene hydroxylase activity; 4) determination of the retention of an inert dust following chronic lead exposure.

Lead exposure was carried out by the evaporation of lead oxide at approximately 700° centigrade and subsequent condensation of the fine lead particles in the cooling air current. Lead concentrations were measured with the aid of membrane filters and subsequent atom absorption photometry.

Electron microscopic studies of the lung parenchyma

The electron microscopic investigations were carried out on rats having inhaled lead oxide at an average concentration of 150 $\mu g/m^3$ for four days. The lungs were fixed with glutaraldehyde according to the instillation method and embedded in araldite. The alveolar macrophages showed partly discrete, partly pronounced alterations especially of the endoplasmatic reticulum. The normally parallel arrangement of the reticular membranes changes into a vapid structure showing cisternae-like bulgings with concomitant detachment of the ribosomes. With increasing damage the membranes transform into myelin-like bands, which may extend to the external nuclear membranes in a striking manner (Fig 1). At the same time alterations of the mitochondria can be observed which are characterized by the condensation and clotting of internal structures. The lysosomes, in contrast, exhibit no damage even though in a number of organelles a myelin-like transformation of the lysosomal matrix is noticeable (Fig 2).

The ultrastructure of the epithelial lining of the lead-exposed lung cannot be definitely characterized by morphological description. However, it can be said principally that numerous young cell forms are to be observed besides damaged cells. As an instance, there are cells whose epithelial extensions detach from the basal membrane, projecting freely into the alveolar lumen. The cytoplasm of these cells contains some clotted electron-opaque mitochondria. On the other hand, cells are also observed containing exceptionely numerous reticular membranes (Fig 3). It is striking that many of the pneumocytes type I super-impose their extensions over the apical poles of type II cells. Under these extentions numerous osmiophilic bodies collect which are regarded as secretion products of type II cells (Fig 4). Another striking feature are cells which are situated for the greatest part underneath the pneumocytes type I and which break through the epithelium with regular, coniform extensions. The cytoplasm of these cells is characterized by abundant tonofibrils. However, mitochondria and other cytoorganelles are sparse (Fig 5). According to localization and behaviour we are obviously dealing with young cells which are migrating to the alveolar wall.

The ultrastructural findings permit the conclusion

Exp. group	No. labelled type I-cells/ 1000 cells	No. labelled type I-cells/cm^2 inner lung surface
Control	3,2	$1,5 \cdot 10^5$
Pb	15,5	$9,1 \cdot 10^5$

Tab 1

	Inhalation time weeks	Treatment TiO$_2$-conc. mg/m^3	Lead-conc. µg/m^3	Retention TiO$_2$ mg/Lung	S	Increase retention percentage/control
I c	15	18,8	0	0,40	0,10	
I Pb	15	18,8	82	0,53	0,10	33
II c	15	27,7	0	0,69	0,18	
II Pb	15	27,7	168	0,96	0,29	39

Tab 2

that the alveolar macrophages as well as the pneumocytes type I in the alveolar wall are altered following inhalation of a aerosol as compared to normal animals.

Determination of the H-3-thymidine index of alveolar cells

In the second experimental series mice inhaled the same lead concentration of 150/ug/m^3 for 14 days. 1 h before sacrifice the animals were injected 100/u Ci H-3-thymidine/kg body weight. 1/u thick sections of the araldite embedded lungs were coated with a L 4 emulsion, the autoradiograms developed after 14 days, and the sections stained with toluidineblue-azur II. The labelled cells were counted and related to 1000 unlabelled cells or a unit area of the inner lung surface. Tab. 1 shows the increase of labelled epithelial type I cells in the lead exposed animals. There was no change of the labelling index of the pneumocytes type II.

Determination of the benzopyrene hydroxylase in alveolar macrophages

In a third test series guinea pigs inhaled lead oxide of an average concentration of 150/ug/m^3 for 4 days. The alveolar macrophages of these animals were collected by pulmonary lavage, and the ability of the cells to metabolize benzo(a)pyrene was determined by means of a method by DIAMOND (4) modified by DEHNEN et al. (3). Immediately after inhalation a drastic decrease of activity to 10% of the original value is found. After an inhalation-free interval of 3 days the activity returns to control values.

Influence of lead inhalation on lung clearance capacity for inert dusts

In a fourth experimental series four groups of rats in-

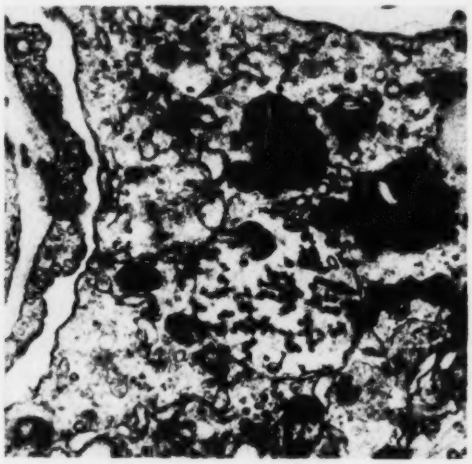

Fig. 1 - Alveolar macrophage of lead-exposed rat. Bulging of endoplasmic reticulum with detachment of ribosomes (arrow). Large phago lysosomes apparently containing lead. 1:25 000

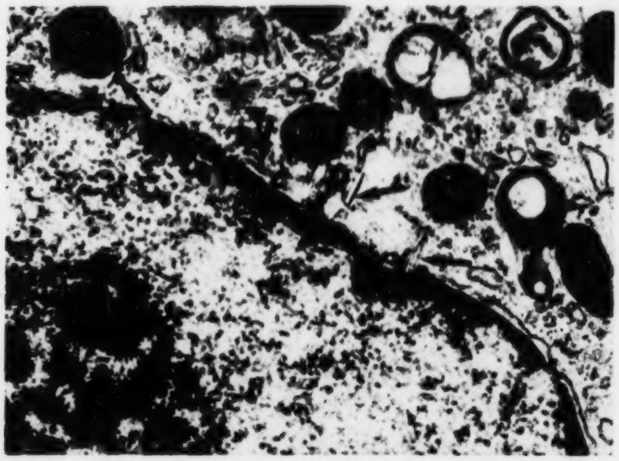

Fig. 2 - Lead-exposed alveolar macrophage. Myelin-like transformation of endoplasmic reticulum (arrow). 1:20 000

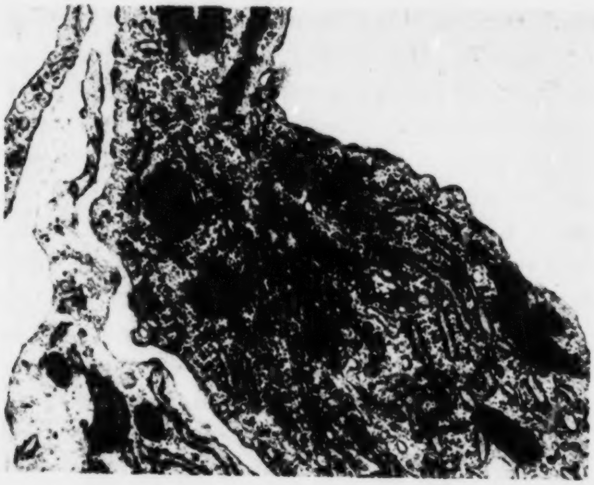

Fig. 3 - Lead-exposed pneumocyte type I with exceptionally well developed endoplasmic reticulum. Some mitochondria (arrow) exhibit condensed matrix and rarification of cristae intermitochondriales. 1:30 000

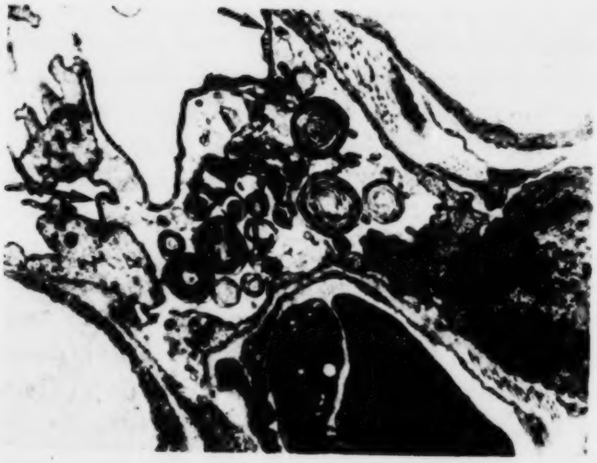

Fig. 4 - Extension of pneumocyte type I situated over the apical pole of a pneumocyte type II (P2). 1:10 000

haled titan dioxide dust in a concentration of 19 or 28 mg/m^3 on 5 days of the week for 3 h. The dispersion of the titan dioxide was performed with a Polly's generator. Each day the fine dust concentration was determined gravimetrically and, in addition, each hour by tyndalloscopy. Two of the groups inhaled a lead aerosol of 82 or 168 $\mu g/m^3$ for 5 h before the exposure to inert dust, while the other two groups were not treated further and served as controls. The inhalations were carried out for 15 weeks in the described way, then the animals were killed, and lungs and lymph nodes were examined for their content of titan dioxide. The results of the analyses are presented in Tab. 2 together with the inhalation data for all four groups. In the lungs of the animals exposed to additional lead inhalation, significantly higher dust quantities were found as compared with controls. The elimination was impaired by 33 to 39%. With regard to the lymph nodes no statistically significant differences were revealed.

Discussion

The effects on the haemopoetic system and the central nerve system have so far been in the foreground of the discussion of toxic lead action. Our investigations demonstrate local effects in the pulmonary parenchyma following inhalation of lead aerosols. Both alveolar macrophages and epithelial cells are affected by the toxic action, as demonstrated by a decrease in enzyme activity in alveolar macrophages and an elevated turn-over of type I cells. The biochemical and morphological findings indicate that the primary point of attack of lead on the cellular level is observed at the reticular membranes. This ist in accordance with studies by SCOPPA and collaborators (5) who could demonstrate a reduction of the activity of membrane-bound hydroxylases in liver cells

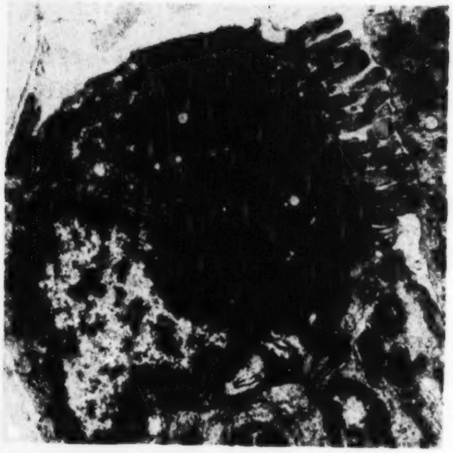

Fig. 5 - Young cell form in the alveolar wall mostly covered by theneighbouring cells. Coniform protrusions break through the epithelial lining. 1:12 000

after oral lead poisoning. As result of the cellular damage an impairment of lung clearance is found. Thus an increased retention of exogenous substances must be expected following lead inhalation. Of further special interest in the context of air pollution is the result that carcinogenous hydrocarbons are metabolized to a lesser degree, thus increasing considerably the period of time these substances are present in the lung. The concentrations chosen by us (70 to 170 $\mu g/m^3$) are slightly lower than the MAK values valid in the Federal Republik of Germany but higher than the values normally measured in our cities. In some cases, however, we measured 24 h averages of 20 to 80 $\mu g/m^3$ in the atmosphere of a West German city in the neighbourhood of a lead plant (2). The pronounced decrease of benzopyrene hydroxylase and the considerable increase of labelled cells in the alveolar wall give rise to the supposition that even clearly lower concentrations than 70 $\mu g/m^3$ can lead to local injury in the lung. The experiments are carried on in this direction.

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DISCUSSION

LYNAM (U.S.A.)

The speaker stated that air lead concentrations in cities average 20 - 80 $\mu g/m^3$. I know of no data to support such figures. In a study of air lead concentrations in seven major US cities at 59 sampling sites, the annual average airborne lead concentrations measured for 24 hours a day for 1 year ranged from a low of 0.17/ug/m3 in a rural community in Los Alamos, New Mexico to a high of 4.4/ug/m3 in Pasadena, California. In a study reported by Prof. Boudène, levels in Paris, France of 2 - 3/ug/m3 are reported. Data by Dr. Tsuchiya in Japan indicate annual levels of lead in air in Tokyo of less than 2/ug/m3. Data from the USEPA CAMP (Continuous Air Monitoring Program) indicate lead levels in cities of a maximum of 1 - 4/ug/m3. Could the speaker support his figure of 20 - 80/ug/m3 average in lead concentrations in cities?

BRUCH (Federal Republic of Germany)

It is correct that the annual concentrations of lead observed in cities average 2 - 3/ug/m3. However, annual averages of 8.3 - 11.9/ug/m3 were noted in areas of high traffic density (1). The concentrations of 20 - 80/ug/m3 we quoted are for 24-hour levels measured by us on particular days in highly industrialized areas (2). Similar or higher levels were reported from other areas having a lead industry (3) (Maximum level: 328/ug/m3; half-yearly average: 50/ug/m3). Since our findings with respect to animals were obtained in some cases after only a few days' exposure, the 24-hour levels seemed to us to offer a more suitable basis for discussion than the yearly average.

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LOW LEVEL LEAD TOXICITY

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ABSTRACT

Lead poisoning in children is associated with several serious pathological and behavioral deficits. Studies in adult animals have been unable to support the association between lead intoxication and behavioral dysfunction. However, when suckling mice are exposed to lead from birth they exhibit an increase in spontaneous motor activity. In this study, mice were exposed to lead acetate (2,5 or 10mg/ml) from birth indirectly through their mothers' milk. At these doses, no classical signs associated with overt lead toxicity were observed. However, growth of the offspring was decreased 10% and in measurements of spontaneous motor activity from 30-150 days of age, the lead intoxicated mice were more than three times as active as coetaneous controls. To elucidate the basic mechanisms underlying this lead-induced behavioral dysfunction, a combined pharmacological and neurochemical approach has been used. The results suggest a defect in the interactions of central cholinergic and aminergic pathways. This study emphasizes the necessity to examine effects of potentially toxic compounds during critical stages of neurological development in order to assess fully the impact of these compounds on humans.

This project was supported in part by the National Institutes of Environmental Health Sciences grants 00034 and 00454 and a Starter Grant to EKS by the Pharmaceutical Manufacturers Association Foundation. EKS is a Joseph P. Kennedy, Jr. Fellow in Neurosciences.

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Clinical studies have documented a relationship between lead intoxication and behavioral disorders in children (Byers and Lord [1]; Chisolm and Harrison, [2]; De la Burdee and Choate [3]; David, et al, [4]). These disorders have been described as irritability, restlessness and agressiveness. Studies in adult animal models have not reproduced these findings which supports clinical knowledge on the relationship between age and susceptibility to lead. Recently, it has been demonstrated that animals exposed to inorganic lead during critical periods of neurological development (analogous to early childhood exposure via pica) develop the behavior disorder of hyperactivity (Silbergeld and Goldberg, [5,6,7]; Sauerhoff and Michaelson, [8]; Allen, et al, [9]). Hyperactivity is seen in animals exposed to levels of lead below that which produces severe growth retardation, kidney damage, paraplegia, or convulsions.

THE ANIMAL MODEL - The intention of this animal model is to provide an analogue to childhood lead poisoning in terms of equivalent age, gastrointestinal route and chronic nature of low level lead exposure. Inorganic lead (2,5, or 10 mg/ml lead acetate) was administered to the mother in the drinking water and through her to the nursing offspring which were the subjects of study. These exposure levels of lead produced a dose-related reduction of growth (about 10% at 5 mg/ml) and a retardation of several developmental landmarks (Table 1). The offspring have no signs of acute lead poisoning but in analyses conducted in collaboration with Dr. J.J. Chisolm. alterations in heme synthesis were found associated with a blood lead level of 50-80 μg%.

TABLE 1. DEVELOPMENTAL LANDMARKS IN CONTROL AND LEAD-TREATED MICE[a]

	Day of Occurrence	
Landmark	Controls	Lead
Eye Opening	13	17
Full Incidence of Hair	16	20
Coordinated Walking	18	25
Weaning	22	30

[a] Data from Silbergeld and Goldberg, [5].

Between 30 and 150 days of age, these animals were individually tested for levels of spontaneous motor activity. In all cases, the lead-treated animals were about 3 times as active as coetaneous controls (Figure 1).

A number of points should be made about this animal model. Increases in the level of lead exposure do not increase hyperactivity but induce other sequelae of lead poisoning. Animals whose lead exposure terminates at weaning also evince hyperactivity as adults. However, animals exposed to lead only post-weaning do not develop the behavioral dysfunction, suggesting that the behavioral toxicity of lead is dependent upon the accessability and immaturity of the developing nervous system.

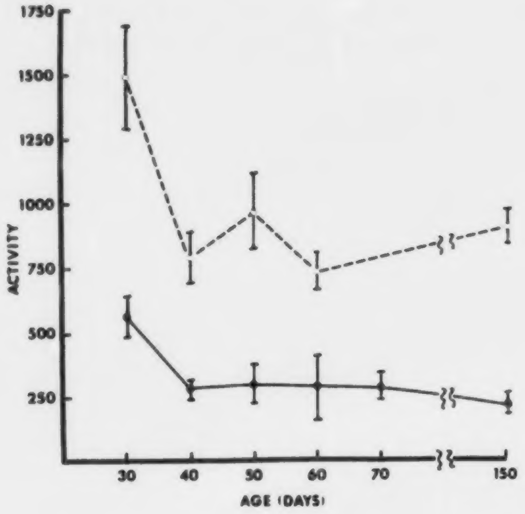

FIGURE 1.

Spontaneous motor activity (counts per hour) of lead-treated and control mice from 30 to 150 days of age. Activity was individually measured electronically; results are shown for second hour of measurement. Open circles and dotted lines indicate results of testing lead treated animals; closed circles and solid lines indicate controls. Points are means of 6-21 animals; vertical lines are S.E.M. The activity levels of treated mice were significantly higher at each day. ($p<0.001$).

Two major approaches were used to study lead-induced hyperactivity. In the first, the behavioral effects of drugs with known mechanisms of action were investigated. Alterations in behavioral response to these drugs may indicate specific changes in neurochemical pathways following lead treatment. In the second approach, neurochemistry of lead-treated hyperactive animals was studied by measuring transport and steady-state levels of neuroactive compounds.

PHARMACOLOGY - Lead-treated hyperactive mice were found to respond behaviorally with marked differences as compared to controls to a number of pharmacological agents (Table 2). The first observation to be made is that lead-induced hyperactivity provides an animal model strikingly similar in its pharmacology to Minimal Brain Dysfunction hyperactivity in children. In addition, a general observation can be made that drugs which act to increase CNS catecholamine-receptor stimulation exacerbate lead-induced hyperactivity (l-dopa, benztropine, and apomorphine). Drugs which are thought to enhance central cholinergic function suppress lead-induced hyperactivity (physostigmine, dimethylaminoethanol, and oxotremorine). Also, the cholinergic antagonists, atropine and benztropine tend to exacerbate the hyperactivity or reactivity of lead-treated animals.

However, several drugs which have been proposed as catecholaminergic agonists do not exacerbate lead-induced hyperactivity but rather suppress it. Amphetamine, methylphenidate and fenfluramine are drugs which enhance catecholamine function by either increasing transmitter release or blocking reuptake. The apparently contradictory responses of lead-treated hyperactive mice to these agents may be partly explained by their action on non-aminergic sites. Amphetamine, in addition to stimulating aminergic pathways can also enhance cholinergic function.

Table 2.

EFFECTS OF DRUGS ON LEAD-INDUCED HYPERACTIVITY a)

DRUG (Dose mg/kg)	EFFECT ON LEAD-TREATED HYPER-ACTIVE CONTROLS		COMMENTS
α-methylparatyrosine (50)	--b)	0	In treated, produces "normal" response to amphetamine; in controls, abolishes amphetamine response
d-amphetamine (10)	--	++	In controls, some stereotypy
dimethylaminoethanol (200)	--	+	
fenfluramine (20)	--	+	
methylphenidate (25, 40)	--	++	
oxotremorine (0.08)	-	0	
physostigmine (0.1-0.5)	--	0	
apomorphine (10)	0	-	In treated, induces stereotypic circling and increases reactivity
atropine (0.01)	0	0	In treated, increases reactivity
benztropine (15)	+	0	In treated, increases reactivity
l-dopa (50)	++	0	In treated, increases reactivity
phenobarbital (20)	++	0	

---

a) Some of these data are reported in Silbergeld and Goldberg, (6,7). The rest is presented in material submitted for publication.

b) +=increase in activity; -=decrease in activité; 0=no change. Number of symbols denotes qualitative amount of change; -- in treated=suppression of activity to that of predrug baseline of control animals.

It is not presently known if the other aminergic agonists methylphenidate and fenfluramine act similarly on the cholinergic system.

NEUROCHEMISTRY - The transport of neurochemicals into neurons has been proposed as an important step in the regulation and turnover of suspected neurotransmitters and may be a valuable method for studying CNS function (Kuhar, [10]). High affinity transport systems are observed experimentally by the low concentrations against which neural tissue will accumulate the compounds. Thus, changes in high affinity transport may reflect alterations in neurotransmitter function. Many of the drugs found to have differential effects on lead-induced hyperactivity are known to affect the high affinity transport of the catecholamines and acetylcholine. In 40-60 day old lead-induced hyperactive mice the high affinity transport of dopamine, choline and tyrosine were significantly affected (Table 3). The transport of other suspected neurotransmitters and a non-neuroactive compound, leucine, were not changed.

The steady state levels of acetylcholine, norepinephrine, and dopamine were also measured in hyperactive animals. Preliminary results indicate that only norepinephrine levels are changed (25% increase). This is consistent with the increases in the transport of tyrosine, a precursor of norepinephrine, and with the suppression of lead-induced hyperactivity by α-methylparatyrosine, a compound which inhibits the synthesis of norepinephrine (Table 2).

CONCLUSIONS - Low level lead exposure has been shown to interfere with central nervous system function and to result in retardation of development, in hyperactivity, and in alterations in response to many pharmacological agents. The mechanism of action is probably related to an inhibition of central

TABLE 3.

HIGH AFFINITY TRANSPORT OF NEUROTRANSMITTERS AND OTHER COMPOUND BY BRAIN TISSUE[a]

COMPOUND	FINAL CONCENTRATION IN ASSAY MEDIUM	N[b]	LEAD HYPERACTIVE AS % OF CONTROLS
H^3-choline	4×10^{-7}M	8	52%[c]
H^3-dopamine	1.25×10^{-6}M	15	81%[c]
H^3-GABA	2.0×10^{-7}M	9	98%
C^{14}-glycine	7.05×10^{-6}M	6	95%
H^3-5-hydroxy-tryptamine	4.67×10^{-8}M	6	105%
C^{14}-leucine	4.3×10^{-6}M	6	88%
H^3-norepinephrine	1.06×10^{-6}M	5	99%
C^{14}-tyrosine	3.3×10^{-6}M	8	115%[c]

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[a] Details of Methodology and Results are in Material Submitted for Publication.

[b] N=number of animals assayed in each group.

[c] $p<0.01$, by Student's T test of the means for control and treated groups.

cholinergic neurotransmission and concomitant alterations in catecholamine pathways. Cholinergic inhibition by lead has been demonstrated at the ganglion (Kostial and Vouk [11]) and at the myoneural junction (Silbergeld, et al, [12,13]).

This animal model lends experimental evidence to support recent findings that some cases of MBD hyperactivity are associated with early exposure to lead (David, et al, [4]). However, the question remains as to the significance of lead exposure in the etiology of MBD hyperactivity.

The enhanced susceptibility of young children to the effects of low level chronic lead exposure has been reproduced in this animal model. This susceptibility may directly result from the immaturity and vulnerability of the developing CNS. In young rodents, the blood brain barrier is more readily permeable than in older animals. Additionally, neurotransmitter pathways are rapidly changing in amounts of neurotransmitters as well as in activities of important metabolic enzymes (Benjamins and McKhann, [14]). Therefore, it is important to carefully choose from populations at risk those groups likely to possess increased vulnerability in order to assess fully the potential hazard from environmental agents.

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DISCUSSION

KAMINSKI (U.S.A.)

1) What were the dose levels in the figures shown in your presentation?

2) What was the lowest level at which you were able to observe changes?

3) Since the highest level i.e. 10 mg/ml is equivalent to a lead intake in a 60 lb child of 50g per day do you consider this to be low lead level?

4) Even the 5 mg/ml dose level of lead is 5000 times the normal lead intake in our diet, this hardly is a low lead level.

GOLDBERG (U.S.A)

1) In the manuscript all doses are indicated. The data shown for the developmental landmark slide and the first drug slide (amphetamines) relate to animals from the 10mg/ml dose. All the other data were from groups exposed to 5mg/ml.

2) The lowest dose we have used is 2mg/ml. This dose produces hyperactive animals.

3-4) One cannot directly compare exposure in animals and exposure in man. Let me point out that these animals do not exhibit the classical signs of lead toxicity and have a blood lead level of 50-80 µg %. At the present time I would have to consider that these animals are at low-level exposure.

TER HAAR (U.S.A.)

If your idea of hyperactivity is correct, why are there not evidences of increased hyperactivity in the large group of children in the United States who have blood leads in the 50-80 mg/100ml range? While there is speculation in the literature that an increase from 25-30 mg lead/100ml may cause hyperactivity, there is no evidence of this fact in the large group of children in the higher lead in blood level.

GOLDBERG (U.S.A.)

At this point in time I would not conclude that lead is etiologic in hyperactivity but I would pose it as a question. Obviously there are many other factors that one would have to consider. Let me point out that the hyperactivity seen in animals may be the first sign of lead poisoning and that other sequelae develop at higher exposure.

STUDIES OF LEAD ENCEPHALOPATHY IN THE DEVELOPING RAT

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ABSTRACT

Inorganic lead produces cerebral dysfunction and clinically definable encephalopathies in man. The expected sequelae of pediatric lead encephalopathy consist of altered behavioral patterns and learning disabilities. To date there have been few studies on the biochemical changes in brain following exposure to inorganic lead. Studies correlating toxicity to behavioral and brain neurochemical changes following lead exposure have been hindered because adult laboratory animals are resistant to the central nervous system (CNS) effects of lead poisoning. Such studies have been impeded by lack of suitable experimental models until Pentschew and Garro(1) *showed that brain lesions develop in neonatal rats when a pregnant rat newly delivered of her litter is placed on 4.0% lead carbonate containing diet. Lead passes into the developing sucklings via maternal milk. Lead poisoned newborns have pronounced retardation of growth and during the fourth week of life develop paraplegia and severe signs of lead encephalopathy, namely extensive histological lesions of the cerebellum and brain edema.*

We have employed this experimental model to study both severe and mild forms of lead encephalopathy in the developing rat. In the former we have found a failure of cell multiplication in the cerebellum. However, in the latter we have found that during the fourth week of development young rats display hyperactivity,

tremors and stereotype behavioral patterns. Pair-fed controls coetaneous to experimental groups do not display such activities. Estimation of brain serotonin, gamma-amino-butyric acid, norepinephrine and dopamine suggests a disruption in catecholamine metabolism during periods of altered behavior in lead exposed developing rats. The data indicate that under conditions of chronic ingestion of relatively low levels of lead from birth to adulthood brain dopamine metabolism is either unchanged or slightly slowed. On the other hand, norepinephrine metabolism is increased by as much as 30% and leaves the brain (turnover time) more rapidly than in control animals.

It is possible that our findings on increased motor activity and changes in brain monoamine metabolism may correspond to early responses to lead exposure before recognized overt signs of toxicity.

The brain is exceptionally sensitive to the effects of inorganic lead poisoning and it is primarily the young - from birth to about seven years of age - who show the most serious damage following symptomatic lead poisoning. Brain manifestations of lead induced toxic episodes and cerebral related sequelae of pediatric lead encephalopathy are well defined and there is considerable knowledge about the histopathological conditions of brain tissue of those lead poisoned individuals who do not survive. Our laboratory raises three questions: (1) is there a neurochemical basis for the cerebral and behavioral deficit as seen in some survivors of lead encephalopathy and more important (2) whether a long standing minor degree of elevation of blood lead concentration would be associated with future neurologic malfunction and (3) is impaired brain function associated with lead ingestion which is asymptomatic or produces symptoms less severe than encephalitis?

Until recently experimental studies on lead encephalopathy has been hampered by the inability to produce in laboratory animals unequivocal neuropathological or behavioral changes characteristic of the human disease. Most adult experimental animals do not display manifestations of brain dysfunction as usually seen in children with lead poisoning. This is not the case when young animals are used. Experimental lead encephalopathy, with histologic features characteristic of the human disease [1,2], can be produced in suckling rats by feeding diet containing 4.5% lead acetate to lactating mother rats after delivery of her young. The lead is transmitted to the newborns through the mother's milk which contains lead. While the mother rats tolerate the diet [1] the sucklings develop paraplegia and extensive vascular damage to the cerebellum, frequently leading to death.

We have used this model to study the biochemical interaction of lead with brain to seek a chemical basis for behavioral aspects of lead poisoning as it relates to the CNS. We found that suckling rats ingesting lead via the milk of mothers fed lead containing diet results in a pronounced retardation in growth of the young and gross discolorization of the cerebellum as previously described [1,2]. There was an apparent reduction in brain weights in the lead exposed animals and a diminution in DNA content of the cerebellum suggesting failure in cell multiplication and/or maturation in this organ. This appears to represent a working experimental model of the more severe and fulminating forms of lead encephalopathy but is much too destructive for the answer to the earlier question: Are there brain effects associated with low levels of lead ingestion which are asymptomatic or which produce symptoms less severe than encephalitis or usually looked for signs of lead poisoning?

Our initial finding suggested that low body weights of animals suckling from mothers fed lead acetate and the cerebellar changes were similar to those seen in undernutrition. Furthermore, it was observed that the appearance of paraplegia nad cerebellar pigmentation occurred shortly after the time when the neonates were capable of gaining access to the mothers' 4.5% lead acetate containing diet [3].

The question was raised whether access to the relatively greater amounts of lead contained in the maternal solid food (24,000 ppm Pb) compared to maternal milk could account for the histopathology observed in reports using this regimen [1,2,3]. We devised a means of sampling the maternal milk for estimation of its lead content. Figure 1 demonstrates

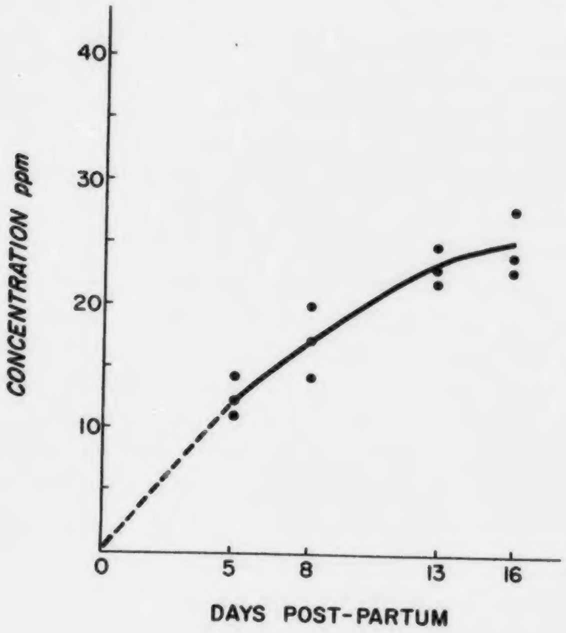


FIG. 1 Milk lead content (ppm) of lactating rats eating 4.5% (2.4%, 24,570 ppm) lead acetate containing diet. Normal milk contains less than 0.02 ppm Pb.

that a lactating rat eating 4.5% lead acetate (2.4% lead, 24,000 ppm Pb) diet produces milk containing increasingly greater concentrations of lead in relationship to length of exposure and increasing food consumption (not illustrated). By the sixteenth day they produce milk containing approximately 25 ppm lead, whereas milk from lactating rats eating normal diet contains less than 0.02 ppm lead. During suckling the developing rat is exposed to less than one thousandth (1/1000) the amount of lead their mothers had been eating during the first 3 weeks of the nursing period.

Developing newborn rats suckling milk containing 25 ppm lead are capable of climbing into mothers' food jars by 18 days of age and thereby capable of consuming solid diet with a high lead content. In order to keep the lead exposure of the developing rat constant, on the seventeenth day the maternal diet was changed to one containing 25 ppm to 400 pmm lead, comparable to, or sixteen times greater than the amount they had consumed in the milk. There is a consequential acceleration of growth rate due to change from a liquid maternal diet to solid food in both control and experimental animals. There was no paraplegia and brains did not contain pigmentation as observed in earlier studies [1-3]. Neonatal rats exposed to these relatively low levels of dietary lead for the first three weeks of life exhibit hyperactivity, aggressiveness, and stereotype repetitive behavior. At 23, 24 and 25 days of age, complete families of six siblings from pair-fed controls or lead exposed groups were placed on a spontaneous activity metering device for 24 hours. Appropriate pair-fed control and experimental groups were tested on alternate days for a single 24 hour period. Neonatal rats exposed to dietary lead for the first 3 weeks of life exhibit a 40 to 93% greater activity than coetaneous pair-fed controls (Figure 2).

Exposure to lead via the regimen described leads to a significant increase in the amount of lead in brain within 5 days of the onset of exposure (Figure 3). The brain lead content of exposed animals continues to increase but the levels are merely one tenth that found when sucklings are weaned to mothers' 4.5% lead acetate diet [3].

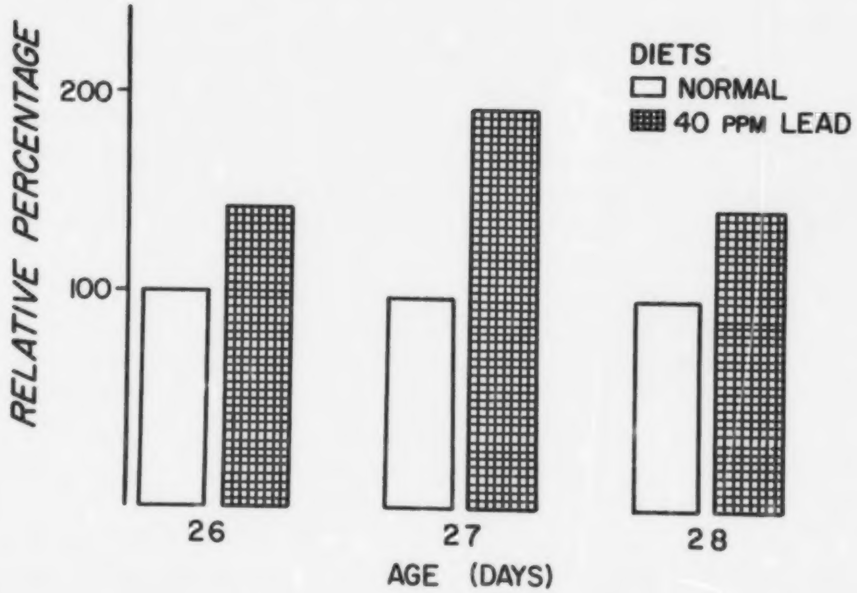


FIG. 2 Percentage spontaneous activity in young lead exposed rats relative to controls (normalized to 100%).

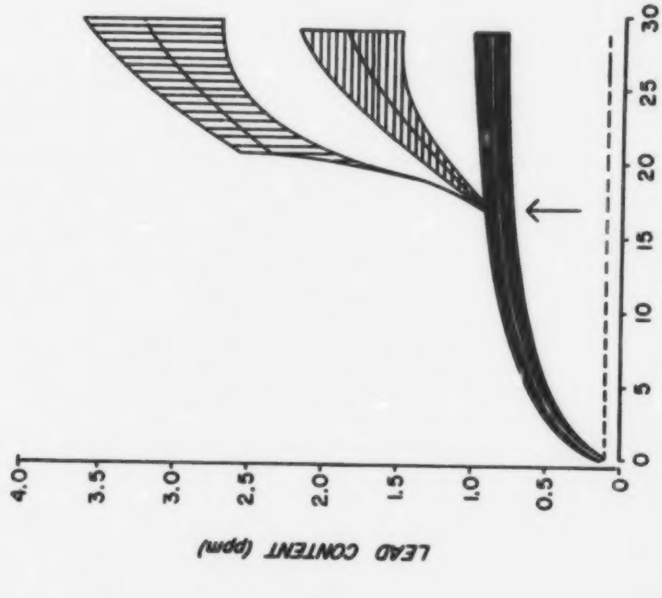


FIG. 3 Brain lead content of neonatal rats suckling mothers eating 4.5% lead acetate diets (0–10) changed (↑) to 40 (■), 400 (▥), and 4,000 (▤) ppm lead containing diet (17–58).

We have now studied the metabolism of brain catecholamines using animals as described above except that sucklings were weaned to a diet containing 400 ppm lead. Experimental and control rats were killed at different ages and their brains analyzed for norepinephrine and dopamine content (Figure 4).

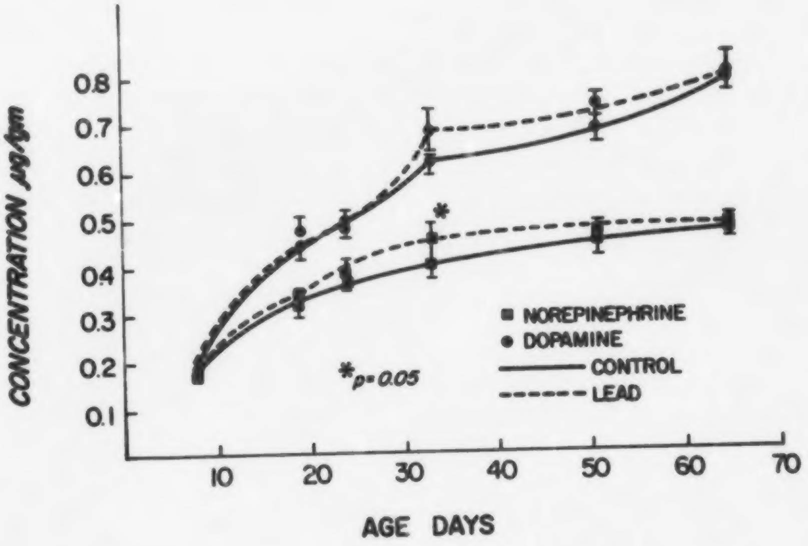


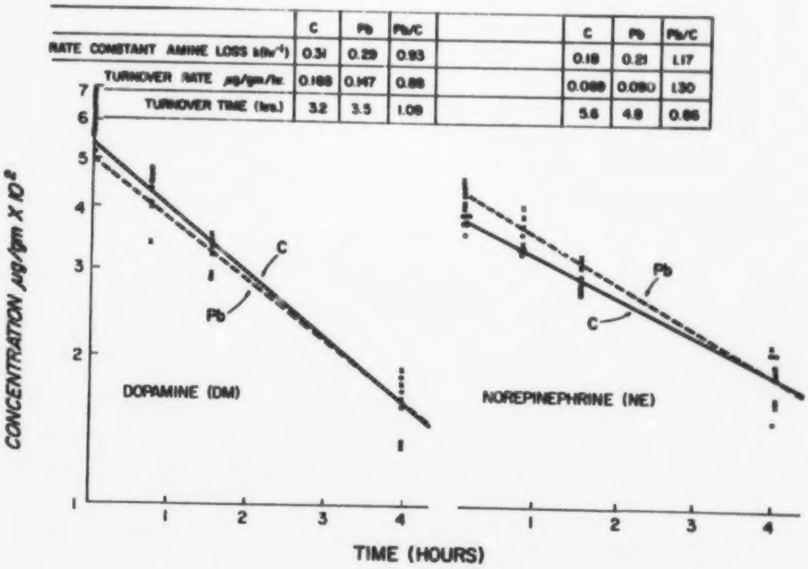
FIG. 4 Norepinephrine and dopamine in brain of leaded and pair-fed control rats.

It is interesting to note that in this study there appears to be slightly higher levels of norepinephrine and dopamine in lead treated rats but the small number of animals employed make difficult a statistical validation of this impression.

It is important to emphasize that this newer mode of developing lead intoxicated rats results in subtle responce (hyperactivity, aggressiveness, stereotype repetitive behavior) and one might suspect similar subtle changes in neurochemical components. Whereas steady state levels of tissue concentration yield static datum; metabolic activity or turnover provides more meaningful data. We have studied norepinephrine and dopamine turnover in brains of lead exposed developing rats. Since these chemicals in brains are thought to be involved in motor activity and behavior, and because earlier studies indicate increased activity in the fourth and fifth week, we examined the turnover of catecholamines in 32 day old rats receiving 400 ppm lead.

We used the drug alpha-methyl tyrosine which is a specific inhibitor of the enzyme tyrosine hydroxylase - the rate limiting step in catecholamine biosynthesis. As the neuronal metabolic activity continues the non-replenished stores of the neurotransmitters are depleted. The rate of depletion is an indication of the neuronal activity.

The data indicates that under conditions of chronic ingestion of relatively low levels of lead from birth to adulthood leading to increases in brain lead content and altered states of behavior, that dopamine metabolism is either unchanged or partially slowed; whereas norepinephrine turnover is increased by as much as 30%, and that norepinephrine leaves the brain 14% more rapidly in leaded animals then in controls (Figure 5).



	C	Pb	Pb/C		C	Pb	Pb/C
RATE CONSTANT AMINE LOSS k(hr⁻¹)	0.31	0.29	0.93		0.18	0.21	1.17
TURNOVER RATE μg/gm/hr.	0.168	0.147	0.88		0.069	0.090	1.30
TURNOVER TIME (hrs.)	3.2	3.5	1.09		5.6	4.8	0.86

FIG. 5 Catecholamine metabolism in brain 32 day lead exposed (....) and control (———) rats receiving alpha-methyl tyrosine, 300 mg/kg.

It is possible that our findings on increased motor activity and changes in brain catecholamine metabolism may correspond to early responses of lead exposure before the usually looked for overt signs of lead toxicity. It may also be that the criterion of what is a toxic level of exposure may have to be redefined.

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DISCUSSION

STARA (U.S.A.)

I want to congratulate Dr. Michaelson and also the previous speaker, Dr. Goldberg, for presenting data which are badly needed to clarify an extremely important question of neurological effects of lead in growing organisms. I have two questions:

1. Why did you not expose the experimental animals through the intra-uterine route - since the major portion of organogenesis of CNS takes place during this period of development?

2. Studies by Kostial et al. in Zagreb, Yugoslavia, show that up to 50% of ingested lead is absorbed during the lactation period in the neonate, later, after weaning, the absorption rate is rapidly reduced. Did you obtain data in these experiments on the absorption rate of lead in time and on tissue burden of Pb, particularly in the brain?

MICHAELSON (U.S.A.)

1. You may be correct about orgnogensis in a general sense but I think you may be somewhat in error about the brain. It is the effects of lead on the central nervous system in which we are interested. The brain of the new born human is relatively

poorly developed and quite immature at birth. Relative to the number of cells in the mature brain fifty percent of cortical cells are present at birth and more significantly only 5 to 7% of the cerebellar cells are present at birth. You therefore have an organ system which is replicating very rapidly in the human from birth to about 24 to 30 months of age. This corresponds to the first three weeks in the newborn rat. This is what makes this experimental model of lead encephalopathy, so interesting and of potential value. Especially if you consider that most human cases of pediatric lead encephalopathv coming to hospitals at less than 7 years of age and indeed about 80% of the cases suffering from bad encephalopathy are less than 4 years of age. You can therefore appreciate that if our concern is the pediatric population then we should look at the neonate and not at the embryo which is an entirely different problem from that the which we address ourselves.

2. Within the time frame of our experiments there is a dose related increase of lead in brain. At the higher exposures where the animals are severlv intoxicated and die the brain contains about 10 to 12 ppm (wet weight) of lead. This is within the range found in younq humans who have died following lead ingestion. In our relativelv lower lead level exposure studies in the absence of the overt signs of bad poisoning and where the animals appear hvperactive there is about a five fold increase in brain lead content over that of controls. Other than the brain we have not studied tissues for their lead concentration other than blood. Both brain and blood levels remain elevated for longer periods following removal of lead from the diet.

SILBERGELD (U.S.A.)

We have begun work on effects of prenatal exposure to lead, and the results suggest that the same decreases in growth and alterations in behaviour as have been reported bv us and bv Dr. Michaelson have also been observed on postnatal models.

EPSTEIN (U.S.A.)

Drs. Goldberg and Michaelson are to be warmlv congratulated on their presentation demonstrating behavioral changes and learning deficits in young rodents exposed to relativelv low levels of lead. Such data are reflected in the recent and proper decision of the EPA to phase out the use of lead as a gasoline additive, a decision which is warmlv endorsed bv the concerned and knowledgeable scientific communities in the USA and elsewhere. The authors have no reason to be diffident about the low levels thev tested especiallv in view of alarming evidence of very high lead levels in street dust and close-to-traffic "corridors."

Apart from the well recognized learning disabilities in children recovering from acute lead poisoning, there have however been no adequate epidemiological studies on learning disabilities in children with blood lead levels in the 60-80 /ug/100g range. Such studies are difficult, because children at risk often live in slums where poverty and other social factors are complicating elements. Nevertheless, occupational studies have recently demonstrated learning disabilities, altered psychological performance and motor nerve conductivity disturbances in man exposed to lead with blood lead levels in only the 60-80 /ug/100g range.

SUBCHRONISCHE ORALE TOXIZITÄT VON CADMIUM BEI RATTE UND HUND

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KURZFASSUNG

Es wurde die subchronische orale Toxizität (90-Tage-Test) von Cadmium bei Ratte und Hund untersucht.

3-monatiger Fütterungsversuch an Ratten *Je 20 männliche und 20 weibliche Ratten erhielten Cadmium (als $CdCl_2$) in Dosierungen von 0, 1, 3, 10 bzw. 30ppm 3 Monate lang täglich mit dem Futter verabreicht.*

Bei den behandelten Ratten waren Aussehen, Verhalten, Futteraufnahme, Wachstum und Sterblichkeit während der 3-monatigen Versuchszeit nicht beeinflusst. Die Verabreichung von Cadmium verursachte keine Blut-, Leber- oder Nierenschädigungen.

Der systolische Blutdruck war bei den behandelten Tieren aller Versuchsgruppen nicht erhöht. Sektionen und histo-pathologische Untersuchungen ergaben keine Hinweise für Schädigungen. Cadmium wurde dosisabhängig in Nieren und Leber gespeichert.

3-monatiger Fütterungsversuch an Hunden *Je 2 männliche und 2 weibliche Hunde erhielten Cadmium (als $CdCl_2$) in Konzentrationen von 0, 1, 3, 10 bzw. 30 ppm 3 Monate lang mit dem Futter verabreicht.*

Bei den behandelten Hunden waren Aussehen, Verhalten, Futteraufnahme, Wachstum und Sterblichkeit in allen Versuchs-

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gruppen nicht verändert. Blut-, Leber- oder Nierenschädigungen wurden nach oralen Cadmiumgaben bis zu 30 ppm im Futter nicht verursacht.

Systolischer und diastolischer Blutdruck waren bei allen Versuchsgruppen bis 30 ppm normal. Sektionen und histo-pathologische Untersuchungen ergaben keine Hinweise für Schädigungen. Cadmium wurde vor allem in Nieren und Lebern dosisabhängig gespeichert.

Dosen bis 30 ppm Cadmium im Futter wurden von Ratten und Hunden 3 Monate lang schädigungslos vertragen.

ABSTRACT

The subchronic oral toxicity (90-day test) of cadmium in rats and dogs was tested.

3-month feeding experiment on rats *Cadmium (as $CdCl_2$) was administered in doses of 0, 1, 3, 10 and 30ppm daily to 20 male and 20 female rats for 3 months with their food.*

During the 3 months of the test, the appearance, behaviour, food intake, growth and mortality rate of the treated rats remained unchanged. The blood, liver and kidneys were unaffected by the cadmium intake.

The systolic pressure did not increase in the treated animals in any of the test groups. Dissections and histopathological tests yielded no evidence of damage. Cadmium accumulated in the kidneys and liver in proportion to the size of dose.

3-month feeding experiment on dogs Cadmium (as $CdCl_2$) was administered in concentrations of 0, 1, 3, 10 and 30ppm for 3 months to 2 male and 2 female dogs with their food.

The appearance, behaviour, food intake, growth and mortality rate of the treated dogs remained unchanged in all the test groups.

The systolic and diastolic pressure in all the test groups up to 30 ppm was normal. Dissection and histopathological tests yielded no evidence of damage. Cadmium accumulated chiefly in the kidneys and liver in proportion to the size of dose.

Rats and dogs withstood doses of up to 30ppm of cadmium with their food for 3 months without damage.

1. Einleitung

Schließt man die gewerbetoxikologischen Probleme beim Kontakt mit Cadmium aus der Betrachtung aus, so wird dieses Metall praktisch nur oral vom Menschen aufgenommen. Es ist daher erforderlich, toxikologische Untersuchungen zur Abschätzung der möglichen Gefährdung durch den Umgang mit cadmiumhaltigen Bedarfsgegenständen nur bei oraler Verabreichung durchzuführen.

Die in der Literatur beschriebenen Untersuchungen sind entweder nicht mit oraler Applikation durchgeführt worden und/ oder sie entsprechen nicht den Kriterien, die eine Gefährdungsbeurteilung möglich machen. Es erschien deshalb notwendig, weitere Versuche mit oraler Applikation durchzuführen.

2. Ergebnisse

Es wurde die subchronische orale Toxizität (90-Tage-Test) bei Ratte und Hund untersucht.

2.1. Ratten

Je 20 männliche und 20 weibliche Ratten erhielten Cadmium (als $CdCl_2$) in Dosierungen von 1, 3, 10 bzw. 30 ppm 3 Monate lang mit dem Standardfutter verabreicht. Als Kontrollen dienten 40 männliche und 40 weibliche Ratten.

Bei den behandelten Ratten waren Aussehen, Verhalten und Futteraufnahme sowie Sterblichkeit während der 3-monatigen Versuchszeit nicht beeinflußt. Die Gewichtskurven (Abb. 1) der behandelten Tiere verlaufen etwa wie die der Kontrollen.

Die Ergebnisse der bei Versuchsende durchgeführten Blutuntersuchungen lassen erkennen, daß durch die 3-monatige Cadmiumapplikation bis zur Dosis von 30 ppm im Futter bei keinem der Parameter ein schädlicher Einfluß auftrat. Auch im Differentialblutbild wurden keine pathologischen Befunde erhoben.

Die Leberfunktionsprüfungen (Tab. 1) ergaben keinerlei Hinweise auf eine behandlungsbedingte Veränderung. Alle leberspezifischen Enzyme sowie Bilirubin und Serumproteingehalte lagen im Bereich der Norm. In der elektrophoretischen Auftrennung der Serumproteine unterschieden sich die behan-

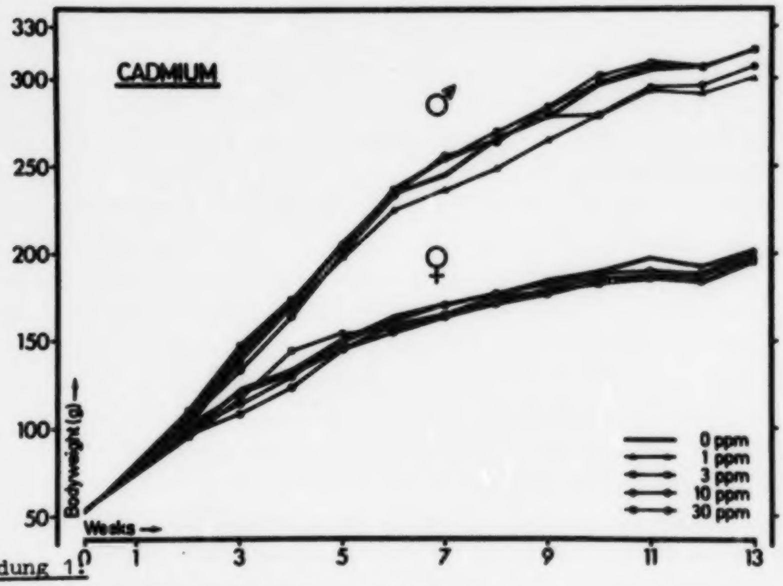

Abbildung 1:

Tiergewichtskurven von Ratten, die 3 Monate lang Cadmium mit dem Futter aufnahmen

Liver-Function-Tests At 3 Month

Dosis ppm	ALP	GOT	GPT	SDH	Total Bilirubine	Total Serum-Protein
	mU/ml				mg/100ml	g/100ml
Male Rats						
0	87,8	27,8	11,5	2,1	0,14	6,6
1	77,6	32,3	14,1	2,7	0,15	6,8
3	83,6	27,6	13,0	2,5	0,20	6,9
10	82,4	34,7	15,6	2,5	0,19	7,1
30	82,9	33,0	18,6	3,1	0,23	7,4
Female Rats						
0	71,3	27,3	13,6	1,7	0,15	6,7
1	83,7	32,5	13,4	2,4	0,20	6,4
3	73,8	29,4	12,0	1,8	0,18	6,9
10	66,9	29,1	14,5	1,8	0,22	6,9
30	86,0	33,8	16,8	2,5	0,19	7,4

Tabelle 1:

Leberfunktionsteste bei Ratten, die 3 Monate lang Cadmium mit dem Futter aufnahmen

delten Ratten nicht von der Kontrolle.
Die Nierenfunktion war bei behandelten Tieren nicht beeinflußt. Die Tab. 2 zeigt die wichtigsten Ergebnisse der Nierenfunktionsteste. Auch hier hatten die behandelten Tiere bis zur höchsten Dosisgruppe von 30 ppm stets physiologische Werte. Blutzucker- und Cholesteringehalte waren bei allen Gruppen im Bereich der Norm. Die Messung des systolischen Blutdrucks (Tab. 3) am Rattenschwanz mittels Gärtner-Manschette und Piezo-Element erbrachte sowohl nach 2- als auch nach 3-monatiger Versuchszeit bei den behandelten Tieren aller Versuchsgruppen keine höheren Werte als bei den Kontrollen. Diese bei 40 Tieren pro Versuchsgruppe durchgeführten Messungen ließen somit keinerlei durch Cadmium bedingte erhöhte Blutdruckwerte erkennen.
Die bei Versuchsende getöteten Tiere wurden eingehend pathologisch-anatomisch untersucht, Hinweise auf Schädigungen ließen sich nicht erkennen. Auch aus den Organgewichten lassen sich keine Schädigungen ableiten. Bei Herz, Nieren, Hypophyse, Gonaden, Harnblase, Uterus, Leber, Milz, Gehirn, Schilddrüsen, Nebennieren, Lungen, Thymus, Magen, Därme, wurden aufgrund von histopathologischen Untersuchungen bis zur höchsten Dosisgruppe von 30 ppm im Futter keine Organschädigungen festgestellt.
Cadmium wurde polarographisch in Leber, Nieren, Harn und Faeces nach 1-, 2- und 3-monatiger Versuchszeit bestimmt. Die Cadmiumgehalte nach 3 Versuchsmonaten (Tab. 4) waren in Leber und Nieren dosisabhängig erhöht. Diese Speicherung ist, wie wir aus Analysen nach 1 und 2 Monaten wissen, ebenfalls abhängig von der Dauer der Applikation. In den Harnen waren dosisunabhängig nur Spuren von Cadmium nachzuweisen, während die Hauptmenge des aufgenommenen Cadmiums in den Faeces erschien.

2.2. Hunde Je 2 weibliche und 2 männliche Hunde erhielten Cadmium als Cadmiumchlorid in Konzentrationen ebenfalls von 0, 1, 3, 10 bzw. 30 ppm 3 Monate lang mit dem Futter verabreicht.
Bei allen behandelten Hunden waren Aussehen, Verhalten,

Kidney-Function-Tests At 3 Month

Dose ppm	Uric acid	Urea	Creatinine	GOT	LAP	Total Protein
	mg/100ml			mU/ml		mg/100ml
	Serum			Urine		
Male Rats						
0	1,3	28,4	1,02	7,1	11,7	24,6
1	1,5	32,4	1,03	5,7	12,5	29,7
3	1,8	31,6	1,05	5,2	13,0	32,7
10	1,8	31,6	1,01	5,7	15,2	30,7
30	2,0	33,3	1,06	7,0	13,3	33,2
Female Rats						
0	1,2	33,2	1,02	4,7	4,4	26,2
1	1,4	33,9	1,04	3,3	4,8	27,7
3	1,6	32,3	1,05	4,1	3,5	28,1
10	1,5	31,9	1,11	3,0	5,2	28,8
30	2,3	36,4	1,14	4,9	5,5	30,7

Tabelle 2:

Nierenfunktionsteste bei Ratten, die Cadmium
3 Monate lang mit dem Futter aufnahmen

Dose ppm	Syst. Blood Pressure (mm Hg), Rat Taile Vein			
	At 2 Month		At 3 Month	
	Male Rats	Female Rats	Male Rats	Female Rats
0	116,9	100,7	121,4	106,2
1	121,9	104,3	123,9	103,8
3	111,7	108,4	121,4	103,1
10	124,3	101,3	125,8	101,1
30	121,9	106,9	121,1	104,2

Tabelle 3:

Systolischer Blutdruck von Ratten, die Cadmium
3 Monate lang mit dem Futter aufnahmen

Dose ppm	Cadmium-Content At 3 Month			
	Liver	Kidneys	Urine (24^h)	Feces (24^h)
	ppm			
Male Rats				
0	0,05	0,13	0,04	1,03
1	0,17	0,49	0,04	0,71
3	0,41	1,06	0,05	1,90
10	3,18	4,98	0,07	11,63
30	8,77	12,06	0,07	46,96
Female Rats				
0	0,05	0,13	0,03	0,69
1	0,59	1,10	0,04	0,87
3	0,39	1,17	0,06	2,55
10	2,96	5,07	0,05	22,87
30	8,91	12,54	0,08	54,13

Tabelle 4:

Cadmiumgehalte in Leber, Nieren, Urin und Faeces bei Ratten, die 3 Monate lang Cadmium mit dem Futter aufnahmen

Futteraufnahme und Sterblichkeit durch die Zumischung von Cadmium zum Futter in allen Versuchsgruppen unverändert. Auch das Wachstum war, wie die Gewichtskurven in Abb. 2 zeigen, unbeeinflußt.

Die eingehenden Bluntuntersuchungen bei allen Hunden (Tab. 5) erbrachten keinerlei Hinweise auf behandlungsbedingte Effekte. Auch im Differentialblutbild waren keinerlei Verschiebungen im Anteil bei den einzelnen Leukozytenarten erkennbar.

Wie aus Tab. 6 hervorgeht, waren die Aktivitäten der leberspezifischen Enzyme in keiner Gruppe dosisabhängig erhöht. Die Bromsulfophthaleinretention lag bei allen Versuchsgruppen im normalen Bereich. Auch Bilirubin- und Gesamteiweiß-Konzentrationen waren nicht verändert. Bei der Serumproteinelektrophorese ergaben sich keinerlei Verschiebungen im Anteil von Albumin und bei den einzelnen Globulinfraktionen.

Die Harnuntersuchungen sowie die klinisch-chemischen Bestimmungen (Tab. 7) zeigten keinerlei schädigende Einflüsse durch die orale Cadmiumgabe. Auch aus Clearance-Totaluntersuchungen von PAH und Inulin lassen sich keinerlei Nierenbeeinflussungen erkennen.

Aus den bei nicht narkotisierten Hunden nach 3-monatiger Versuchszeit durch Arterienpunktion gemessenen Blutdruckwerten (Tab. 8) ergibt sich kein Anhaltspunkt dafür, daß die männlichen oder weiblichen Hunde nach der Cadmiumbehandlung erhöhte Blutdruckwerte aufwiesen.

Aus anatomisch-pathologischen Untersuchungen und aus Organgewichten lassen sich keinerlei Hinweise auf Organschädigungen ableiten. Die eingehenden histopathologischen Untersuchungen bei den in Tab. 9 aufgeführten Organen erbrachten lediglich normale Befunde.

Cadmiumanalysen in Leber, Nieren, Pancreas und Speicheldrüse zeigten dosisabhängig zunehmende Cadmiumgehalte in diesen Organen. Die mittleren Werte finden sich in Tab. 10.

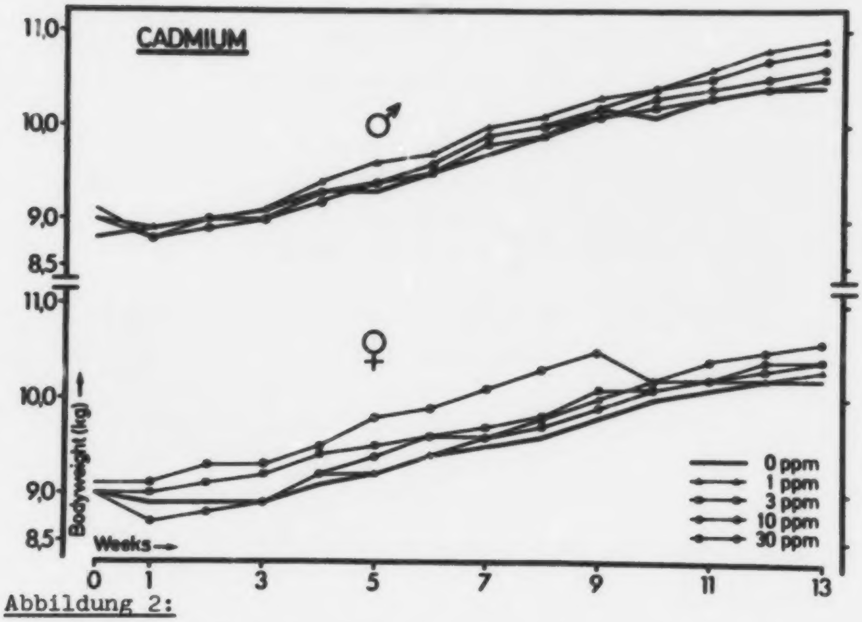

Abbildung 2:

Tiergewichtskurven von Hunden, die Cadmium 3 Monate lang mit dem Futter aufnahmen

Blood Studies At 3 Month

Dose ppm	Sed-rate	Hemogl.	Hematocr.	Ery.	Retics	MCHC	MCV	Leucoc.	Thromboc.	Prothrombintime
	1h/2h	g%	%	10^6	‰	γγ	μm^3	10^3	10^3	sec.
					Male Dogs					
0	1/3	14,7	46	5,92	6	24	78	11	475	8
1	2/4	15,4	45	6,40	6	24	70	9	476	8
3	3/6	15,8	48	6,30	5	25	76	8	380	10
10	1/2	14,9	44	6,12	4	24	72	11	398	9
30	2/5	13,3	40	5,98	6	22	67	11	416	8
					Female Dogs					
0	2/4	16,8	48	7,23	9	23	66	8	532	10
1	1/2	15,8	43	6,27	6	25	69	8	463	10
3	1/3	15,8	46	6,14	5	25	75	7	426	10
10	4/9	15,6	44	6,29	6	25	70	7	476	10
30	1/3	16,4	45	6,72	7	24	67	8	591	10

Tabelle 5:

Blutuntersuchungen bei Hunden, die Cadmium 3 Monate lang mit dem Futter aufnahmen

Liver-Function-Tests At 3 Month

Dose ppm	ALP	GOT	GPT	SDH	LDH	GIDH	BSP	Total Bilirubine	Total Serum-Protein
	mU/ml						mg/100ml		g/100ml
Male Dogs									
0	63	12,4	16,8	1,9	82	1,6	0,20	0,12	6,6
1	50	9,2	7,3	1,4	109	0,8	0,15	0,14	6,7
3	59	9,2	8,4	1,2	73	1,8	0,27	0,12	6,8
10	54	12,3	8,9	0,9	87	1,1	0,25	0,20	6,7
30	56	11,8	7,0	1,3	119	1,0	0,27	0,17	6,6
Female Dogs									
0	64	15,7	6,0	0,7	198	1,6	0,24	0,14	6,3
1	61	10,1	8,1	1,6	56	0,8	0,17	0,15	6,7
3	62	13,7	9,9	0,9	43	0,8	0,22	0,17	6,8
10	68	16,2	10,2	1,0	59	0,7	0,20	0,14	6,6
30	48	14,7	10,6	1,4	142	1,4	0,20	0,21	6,6

Tabelle 6:

Leberfunktionsteste bei Hunden, die 3 Monate lang Cadmium mit dem Futter aufnahmen

Kidney-Function-Tests At 3 Month

Dose ppm	Urea	Uric acid	Crea-tinine	Total Protein
	mg/100 ml			
	Serum			Urine
Male Dogs				
0	18,1	2,0	1,04	25,1
1	19,2	1,7	0,89	24,7
3	16,7	2,0	0,92	32,7
10	16,2	1,9	0,96	25,1
30	15,2	2,0	1,10	36,7
Female Dogs				
0	16,7	1,6	0,93	20,9
1	18,9	1,6	0,97	30,1
3	19,7	1,6	1,10	34,3
10	17,3	1,8	0,87	46,0
30	15,9	1,8	1,28	47,4

Tabelle 7:

Nierenfunktionsteste bei Hunden, die 3 Monate lang Cadmium mit dem Futter aufnahmen

Dose ppm	Syst./Diast. Blood Pressure (mm Hg) At 3 Month	
	Male Dogs	Female Dogs
0	185/123	183/115
1	203/125	190/123
3	178/118	213/135
10	208/135	183/123
30	195/135	193/125

Tabelle 8:

Systolischer und diastolischer Blutdruck von nicht narkotisierten Hunden, die Cadmium 3 Monate lang mit dem Futter aufnahmen

Aorta arch
Lung
Heart
Thymus
Lymph nodes (cervical and mesenteric)
Liver
Gall bladder
Spleen
Pancreas
Kidney
Urinary bladder
Prostate

Gonads
Uterus
Thyroids
Adrenals
Salivary gland
Oesophagus
Stomach (body and antrum)
Duodenum
Jejunum
Ileum
Upper colon
Lower colon

Skeletal muscle
Bone marrow (sternum)
Cerebral cortex
Thalamic nuclei
Mid brain
Cerebellum
Medulla
Central nervous system
Pituitary
Peripheral nerve
Eye
Optic nerve
Cervix
Tonsils

Tabelle 9:

Zusammenstellung der histopathologisch untersuchten Organe

Dose ppm	Cadmium-Content At 3 Month			
	Liver	Kidneys	Pancreas	Salivary gland
	ppm			
Male Dogs				
0	0,14	0,60	0,03	0,05
1	0,24	1,30	0,04	0,08
3	0,51	2,60	0,08	0,12
10	2,67	12,40	0,32	0,16
30	6,21	15,45	0,62	0,36
Female Dogs				
0	0,08	0,38	0,02	0,06
1	0,25	1,45	0,03	0,10
3	0,56	3,40	0,10	0,12
10	2,21	7,19	0,24	0,25
30	6,37	17,15	0,58	0,39

Tabelle 10:

Cadmiumgehalte in Leber, Nieren, Pancreas und Speicheldrüsen bei Hunden, die Cadmium 3 Monate lang mit dem Futter aufnahmen

3. Schlußbetrachtung

Während der Versuchszeit kam es bei den Ratten und Hunden zu zunehmender Speicherung von Cadmium vor allem in Nieren und Lebern. Trotz dieser Speicherung wurden weder die Lebern noch die Nieren morphologisch oder funktionell geschädigt. Auch eine Veränderung des Blutes konnte nicht gefunden werden. Selbst nach Dosen von 30 ppm Cadmium im Futter war sowohl bei der Ratte als auch beim Hund der Blutdruck nicht erhöht. Schädliche Einflüsse auf Reproduktionsorgane wurden nicht beobachtet. Hiermit konnte gezeigt werden, daß bei oraler Gabe Cadmium selbst bis zu Dosierungen von 30 ppm im Futter ohne Schädigungen 3 Monate lang vertragen wurde. Dieses Ergebnis veranlaßte uns, weitere längerdauernde toxikologische Untersuchungen zu starten.

DISKUSSION

PISCATOR (Schweden)

1. Hat man Kadmium und Zink im Standardfutter bestimmt ?

2. Was ist die Norm? Hat man nicht die 30ppm-Gruppen mit Kontrollgruppen verglichen?

LORKE (Bundesrepublik Deutschland)

1. Cadmium wurde im Standardfutter bestimmt. Die Konzentrationen lagen zwischen 0,01 und 0,1 ppm. Zink wurde im Futter nicht bestimmt.

2. Der statistische Vergleich wurde mit der Individualkontrolle durchgeführt. In unserem Institut liegen jedoch auch sämtliche bei vielen anderen Kontrollgruppen bestimmten Werte vor. Daher haben wir einen guten Ueberblick über die normale Variation der untersuchten Parameter bei unsern Versuchstieren. Alle im vorgetragenen Versuch gefundenen Werte lagen innerhalb dieser Norm.

KJELLSTROEM (Schweden)

Die von Ihnen angegebenen Werte für die Cadmium-Konzentration in der Niere waren selbst bei der Gruppe mit der höchsten Belastung um das Zehnfache niedriger als die Pegel, die nach allgemeiner Annahme zu Wirkungen führen. Auch nach Berechnungen auf Grund der Dosis selbst entsprechen die Werte für die tägliche Aufnahme durch den Menschen 100-200 µg pro Tag, also Mengen, für deren Wirksamkeit bisher noch kein Beweis geliefert wurde. Wäre es nicht ratsam, ein Belastungskollektiv einzubeziehen, bei dem sich tatsächlich Auswirkungen zeigten, so dass man weiss, dass Ihre Methoden richtig sind? Warum führten Sie diese Untersuchung durch, wo es doch so viele japanische und andere Untersuchungen gibt, die zeigen, dass man höhere Pegel braucht, um erste Wirkungen zu erzielen?

LORKE (Bundesrepublik Deutschland)

Ihre Meinung, dass 30 ppm Cadmium in der Nahrung schädigungslos vertragen werden, wird allgemein nicht geteilt. In ersten Versuchen wollten wir no-effect-Dosen für Cadmium finden. Weitere Untersuchungen mit höheren Dosen und über längere Zeit sollen uns helfen, auch Ihre Frage klar zu beantworten.

LAUWERIJS (Belgien)

Sie ziehen die Schlussfolgerung, dass bei Tieren, die in ihrer Nahrung 1 bis 30 ppm Cadmium erhielten, keinerlei von Cadmium ausgehende Wirkung festzustellen war.

Mir scheint, aus den in Tabelle 7 dargestellten Ergebnissen geht hervor, dass zumindest bei weiblichen Hunden ein gutes Dosis-Wirkung-Verhältnis zwischen Proteinurie und Cadmium-Exposition besteht.

Könnten Sie diese Ergebnisse etwas erläutern?

LORKE (Bundesrepublik Deutschland)

Eine durch Nierenschädigung bedingte Eiweissausscheidung beginnt oberhalb von 100 mg/100ml Urin. Alle bei unsern Tieren gefundenen Eiweisswerte liegen erheblich niedriger Mit den üblichen Eiweissnachweismethoden (Essigsäure-Kochprobe, Sulfosalicylsäure-Probe) waren sämtliche Urine negativ. Es ist keine Seltenheit, bei unbehandelten nierengesunden Hunden Eiweisskonzentrationen bis zu 100 mg/100ml Urin zu finden. Aus keinem der von uns gefundenen Werte lässt sich eine Nierenschädigung ableiten. Auch die elektrophoretisch durchgeführte Auftrennung ergab keinen Anstieg der niedermolekularen Fraktionen.

CRITERIA FROM ANIMALS EXPOSED TO KNOWN CONCENTRATIONS OF NITROGEN DIOXIDE AND OZONE WITH POTENTIAL USE IN EPIDEMIOLOGY

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ABSTRACT

A realistic concept of the pathogenesis of injury, induced in animals by their residence in ambient concentrations of the oxidizing gas, nitrogen dioxide (NO_2*), has been realized for the rat. Also, observations have been made in the monkey, Macacus speciosa. Physiologic and morphologic changes have been investigated starting immediately upon exposure of young animals and then intermittently during an entire lifetime of exposure of rats. Effects varied from an early significant change in respiratory frequency, during exposure to approximately 0.8ppm of* NO_2*, to the development of a fatal, chronic disease resembling emphysema in man. Associated physiologic sequellae in terms of respiratory function and polycythemia were studied. More recently, similar observations have been made in rats exposed to ambient concentrations of ozone (*O_3*), a more injurious component of smog, and to realistic mixtures of the interdependent oxidizing gases,* NO_2 *and* O_3*. Clinical, pathologic, physiologic and hematologic aspects have been studied in parallel.*

Experimentally derived data will be discussed in relation to detectable events that may logically be anticipated in man exposed to reasonably equivalent circumstances. The problem of selecting appropriate end points, or criteria, quantifiable by

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or in man for epidemiologic purposes will be considered from the viewpoint of responses elecited in animals through controlled experimental studies. Included in the considerations will be criteria for both cigarette-smoking and non-smoking populations because smoking of tobacco is a fairly universal habit. The intended evaluations will be in support of the development of an acceptable spectrum of health-related effects in countries with populations subject to exposure to atmospheres polluted with photochemical oxidants.

Much has been learned about the responses of laboratory animals residing in atmospheres polluted with either nitrogen dioxide (NO_2) (Freeman et al. [1-4]) or ozone (O_3) (Stokinger et al. [5], Werthamer et al. [6], Freeman et al. [7], Schlipköter et al. [19]), but application to the epidemiology of disease in man is difficult. This is due to the complexity of photochemical reactions themselves[8,9], to the variability of man's activities, and to the usual difficulties in devising effective epidemiologic designs.

At least four special criteria are required in grouping individuals for a meaningful epidemiologic study of the effects of oxides of nitrogen (NO_x) and oxidents. The first has to do with residence in crowded, traffic-laden and industrialized areas and alludes to the daily cycling of the toxic, interdependent, photochemically induced atmospheric pollutants, NO_2 and O_3, that occur almost simultaneously during half of each day in varying ratios and in fairly similar average concentrations [8,9]. Thus, epidemiologic observations have necessarily been related to the impact of the mixtures. (Our experience with rats residing in atmospheres containing either NO_2 or O_3 reveals that, at least in relatively short-term experiments, O_3 is about ten to twentyfold more injurious to the lungs than NO_2 (Freeman et al. [10])).

Whereas one may limit atmospheric components selectively to NO_2 and O_3 under laboratory conditions, peroxyacetyl nitrates also exist as photochemical products [8], and independent pollutants, such as sulfer dioxide (SO_2) [11], appear frequently to complicate interpretation.

The universal custom of cigarette-smoking is a second factor in the epidemiology of NO_x-induced disease [12]. Among the injurious components of tobacco smoke, oxides of nitrogen are found in hundreds of parts per million (Haagen-Smit et al. [13]), of which an undetermined portion is NO_2. Of the large dose of NO_x carried into the lungs with each bolus of inhaled smoke, almost all is retained (Freeman et al. [14]). The close correlation between the prevalence of human emphysema and chronic bronchitis with the amount of tobacco smoke inhaled over a period of years [12]is especially noteworthy and is reminiscent of the disease readily induced in rats by exposing them continuously to NO_2 in concentrations probably similar to those in the tobacco smoke that traverses the airways of the smoker [4]. As a corollary, nonsmokers are largely spared from chronic obstructive pulmonary diseases regardless of their ambient environments.

A third, obvious factor is occupation. This concerns confined concentrations of either NO_2 or O_3, or both, independent of concurrent photochemical activity. This applies equally to NO_x produced naturally by bacterial action on crops stored in silos, causative of silo-filler's disease in farmers, and to industrial processes such as welding and the use of explosives in mines. High temperatures are reached in which the oxidation of ubiquitous nitrogen is accelerated to form toxic oxides [9]. Similarly, certain occupational activities create high concentrations of either NO_2 or O_3 not achieved in photochemical reactions (Stokinger [15]).

The fourth factor is individual susceptibility, which includes the age of initial exposure. As a rule, the very young are thought to be poorly defended against exogenously induced disease compared with maturing or mature individuals, but this may not be universal, as we shall see. The elderly also are vulnerable due to the high probability of affliction with cardiopulmonary diseases and possibly to aging itself. Individuals prone to respiratory allergy are particularly responsive to atmospheric irritants. With regard to chronologic age, the type of response to exogenous insults may depend in part on whether an individual was born into a polluted environment or was moved into it later in a virgin state, relative to specific environmental components.

What is the evidence for this? Using a species sensitive to NO_2, four-week-old rats increased their rates of erythropoiesis rapidly and developed polycythemia during continuous exposure to the gas [3]. (Figure 1) In contrast, animals exposed from birth generated red cells at a rate less rapid than that expected of newborn rats when compared with normal litters born in clean air. As a result, such exposed rats were relatively anemic upon attaining seven weeks of age, and contrasted sharply with the polycythemic rats of the same age that were exposed only during the last two of the seven weeks [14].

Also, the mechanical compliance of excised lungs of rats exposed to high subacute concentrations of NO_2 (~15 ppm) from birth was not affected by increasing the frequency of respiration. Lungs of animals of the same age, exposed after they were a month old, developed frequency dependence

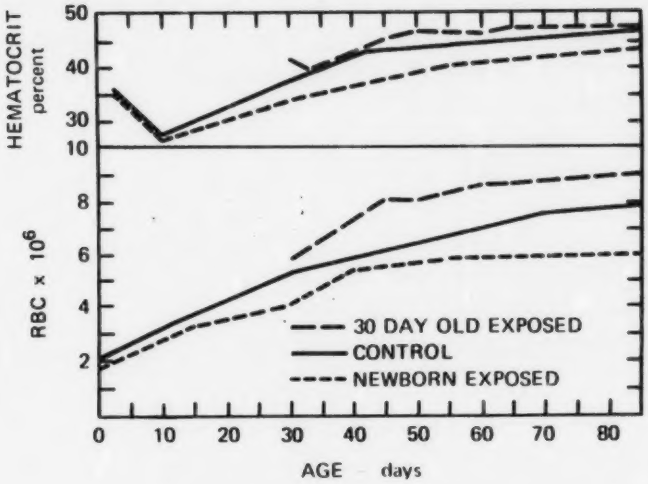

Fig. 1. Erythropoiesis in Immature Rats.

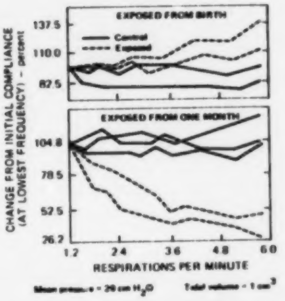

Fig. 2. Frequency-Dependent Compliance (as percent of value at lowest frequency).

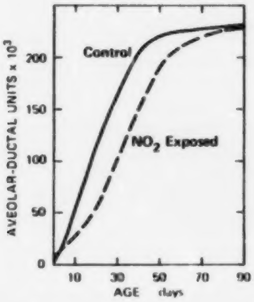

Fig. 3. Total Number of Alveolar and Ductal Air Spaces.

of compliance, presumably due to changes in the small airways [14]. (Figure 2) Incidentally, growth of the lung--defined as the rate of formation of new alveoli in animals exposed continuously to NO_2 from birth--was less than normal for the first ten weeks [14]. (Figure 3) Thus, the failure of animals exposed from birth to have developed small airway disease, as judged by frequency dependence of compliance, may have been determined by neonatal contact with NO_2. Both the hematologic and respiratory observations suggest altered responsiveness, reminiscent of the immunologic tolerance of animals exposed neonatally to antimetabolites (Schwartz et al. [16]). Thus, the significance of very early exposure relative to subsequent pathogenesis is interesting and deserves further study.

Whereas smoking is clearly associated with chronic pulmonary disease in man, ambient ozone has not yet been so identified epidemiologically [8]. What is its impact on the nonsmoking population? Its odor is known to be detectable at a concentration of about 0.02 ppm; increased susceptibility to infection in animals begins at about 0.08 ppm; and eye irritation to peroxyacetyl nitrates is noticeable when the ozone concentration reaches about 0.1 ppm [8]. At that level, increased airway resistance also becomes evident in some individuals, and it is readily apparent when breathing a concentration of about 0.5 ppm in air for three hours on consecutive weekdays. Exposure to an atmosphere of about 1.0 ppm rapidly becomes intolerable to man [8].

In contrast, there is little clinical evidence of effect on the respiratory tract during experimental exposure to NO_2 at levels below 1.0 ppm, although the odor is readily detectable at a concentration of about 0.1 ppm [9]. To simulate maximum human exposure to the mixture of NO_2 and ozone on a cyclic photochemical basis, we exposed rats daily for four hours and observed pathologic effects in the lungs that could relate potentially to chronic pulmonary disease [10]. Using a concentration of about 0.9 ppm of each of the two gases, a lesion developed at the narrow junction of the respiratory bronchiole and alveolar duct through which air is conducted to and from the alveoli. (Figure 4 a,b). Epithelial cells were injured and replaced (Evans et al. [17]), often by hypertrophic cells. The hypertrophic epithelium, supported by fibroblasts and chronic inflammatory cells, proliferated at the level of the smallest airways to form denser tissue. Neither its effect on lung function nor its reversibility

DEVELOPMENT OF LESIONS AT THE NARROW JUNCTION OF THE RESPIRATORY BRONCHIOLE AND ALVEOLAR DUCT EXPOSED TO 0.9 ppm OF NO_2 AND TO 0.9 ppm OF O_3

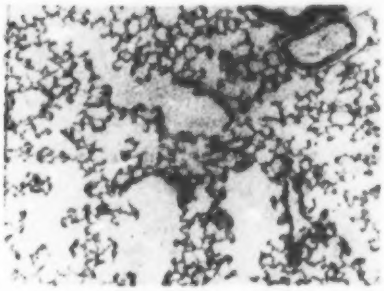

Fig. 4a. Control rat exposed to clean air.

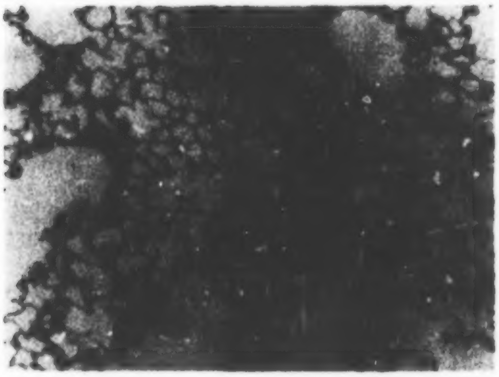

Fig. 4b. Rat exposed to 0.9 ppm of NO_2 and to 0.9 ppm of O_3 simultaneously for four hours daily.

EMPHYSEMATOUS LESION IN LUNGS OF RATS EXPOSED TO 0.9 ppm OF NO_2 AND 0.9 ppm of O_3

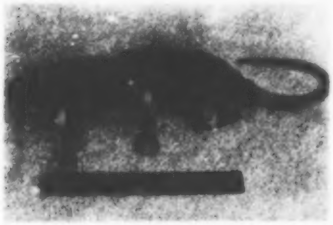

Figure 5a : Normal rat with excised lungs at atmospheric pressure

Figure 5b : Rat after exposure continuously for three weeks to 0.9 of NO_2 and 0.9 ppm of O_3

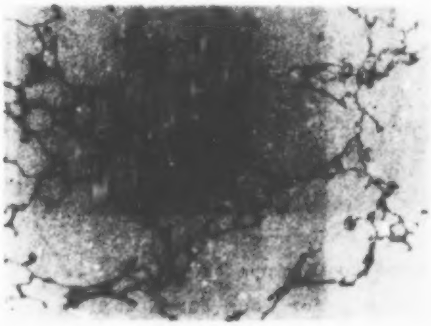

Figure 5c : Emphysematous lesions in lung seen in Figure b

RESPIRATORY BRONCHIOLE AND ALVEOLAR DUCTS IN RATS

Figure 6a : Normal terminal bronchiole and neighboring alveolar duct

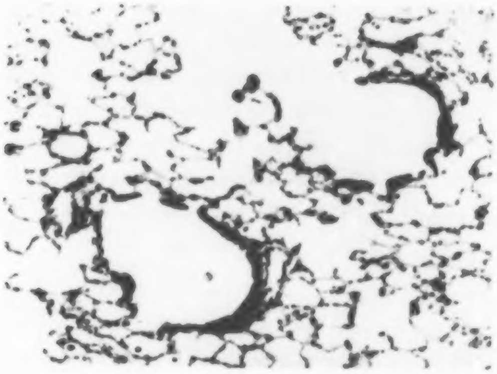

Figure 6b : Epithelial Hypertrophy after exposure to a mixture of 2.5 ppm NO_2 and 0.25 ppm O_3

has been established. As was the case with high subacute concentrations of NO_2 [1,4], with continuous exposure to a mixture of ozone and NO_2--each at a concentration of about 0.9 ppm--rats developed an emphysema-like disease. (Figures 5a, b, c). Also, continuous exposure to a mixture of 2.5 ppm of NO_2 and one-tenth that concentration (0.25 ppm) of ozone resulted in disease of the alveolar ducts characteristic of the response to ozone rather than to NO_2. (Figures 6a, b). Thus, it is clear that ozone is the primary cause of deep-seated pulmonary disease in the experimental animal when mixtures of the agents are presented in concentrations and on a schedule approximating man's experience with smoggy atmospheres. Long-range studies are under way.

Shifting our attention to animal studies with higher subacute concentrations of NO_2, of the order of 14 to 17 ppm [3,4], the issue of smoking and the epidemiology of chronic obstructive pulmonary diseases [12], toward which NO_2 may be contributory, come into focus. Extrapolation to chronic human disease from the rat with a life span of about 4% that of man is hazardous. Nevertheless, the fortuitous similarity in the relative time required for the development of emphysema in the rat, when compared with its development in man, warrants attention [1,3,4]. The clinical and morphological features associated with habitual smoking of cigarettes are consistent with the changes in the lungs of rats exposed to high, subacute concentrations of NO_2 for long periods [1,3,4]. It is noteworthy, also, that ozone, which is absent from tobacco smoke, induces a similar disease in the rat at approximately one-twentieth the concentration of NO_2 when mixed with it in equal amounts [10].

In summary, it would not seem feasible, except in the case of high occupational concentrations and in habitual smoking, to distinguish between the specific effects of NO_2 and O_3, epidemiologically. As photochemically interdependent gases, they must coexist and act almost simultaneously. However, several recent studies suggest that biochemical changes in blood of man (Buckley et al. [18]) may provide a handle by which to correlate the atmospheric presence of these individual pollutants and, possibly, nitric oxide (NO) with systemic effects [14].

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DISCUSSION

STUPFEL (France)

Do you relate the effect you reported on erythropoiesis and the apparition of pulmonary emphysema?

FREEMAN (U.S.A.)

In growing and mature animals, we relate erythropoiesis to the hypoxemia of pulmonary disease (with or without the actual development of emphysema). However, the reference to erythropoiesis in the current presentation is to the difference in response to exposure to NO_2, between neonatal (from birth) rats and more mature neonatal rats. The former fail to achieve the level of erythrogenesis of normal rats in the neonatal state, at least to the age of 90 days, whereas the somewhat more mature neonatal animals are already able to respond with exaggerated erythrogenesis (polycythemia).

Although, it is presumed that the erythrogenic response is very sensitive and reflects some degree of oxygen-want, secondary to the pulmonary effects of NO_2, room must be reserved also for the real possibility that NO_2, or a reaction product, may penetrate the pulmonary-vascular barrier and effect an erythrogenic response on another basis also, for example, by affecting red cells directly or the erythrogenic tissue. At present, there is little or no evidence for this.

STOFFWECHSEL
METABOLISM
METABOLISME
METABOLISMO
METABOLISME

Vorsitzender - Chairman - Président - Presidente - Voorzitter

D. BENINSON (Argentina)

SOURCES AND METABOLIC PATHWAYS OF LEAD IN NORMAL HUMANS

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ABSTRACT

Absorption, excretion and internal distribution of lead has been studied in normal volunteer adult male subjects, maintained under controlled diet and environment for periods of six months in a hospital metabolic unit. Stable isotopes of lead were used to isotopically label lead from food. This permitted distinction of this source from others (atmospheric and internal) as body fluids and tissues exchanged lead internally and with the external environment during the course of the experiments. Lead concentration and isotopic composition in blood, urine, feces, hair, nails, sweat, bile, gastric and pancreatic secretions and bone were determined by mass spectrometric stable isotope dilution analysis.

It was found that the concentration of lead in the blood of these subjects can be simply related to measurable parameters such as the fraction of food lead absorbed in the gut (6 to 14%), the daily quantity of internal lead excreted in urine, hair and secretions into the gut (38 to 50 µg), and the characteristic (e-folding) residence time of lead in the metabolic pool of lead which exchanges rapidly with the blood (∼35 days). Blood lead concentrations could be predictably adjusted within the normal

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range of 0.14 to 0.27 μg/g by controlling the dietary or atmospheric input. Response of the blood lead concentration to removal and introduction of atmospheric exposure to lead at typical urban concentrations of 1 to 2 μg/m^3 showed that about 16 μg/day are added to the blood from this source, which therefore comprised about 1/3 of the daily intake from all sources.

These same techniques were used to study the gastrointestinal absorption of lead. While fasting, absorption factors as high as 50% were found. Differences between individuals and chemical form were also observed.

1. Introduction

The kinetics of the absorption, internal distribution, and excretion of lead at normal concentration levels in normal humans is being studied with the use of stable isotope tracers. The use of these nonradioactive tracers (^{204}Pb, ^{206}Pb, or ^{207}Pb) permits labelling of dietary lead ingested over a particular period of time and permits following the subsequent history of this lead as a consequence of physiological processes. This technique has the advantage of allowing kinetic studies to be carried out under constant, steady-state total lead concentrations, and also minimizes problems of laboratory contamination in chemical analysis, as the sources of lead contamination have isotopic compositions markedly different from those of the isotopic tracers. Other important aspects of the methodology are that all analyses are carried out using the accurate and sensitive technique of mass spectrometric stable isotope dilution, and that the human subjects are maintained on controlled diets and in controlled environments in a hospital metabolic unit. Simultaneous metabolic balance studies are carried out for other elements.

Results obtained on a first subject have been reported previously by Rabinowitz, Wetherill, and Kopple [1] and preliminary results have been given for a second subject [2]. In this work the subject's normal diet was replaced with a low-lead diet. This diet was supplemented with ^{204}Pb, so as to maintain the pre-study level of lead ingestion. In both cases it was found (fig. 1 and 2) that ingestion of a constant quantity of ^{204}Pb nitrate with meals resulted in a rising concentration of this isotope in the blood, which approached a steady state after ~ 100 days. The e-folding time for the increase in ^{204}Pb concentration is ~ 35 days. The increase of concentration of ^{204}Pb in the urine followed that of the blood closely, whereas other fluids (bile, saliva, pancreatic and gastrointestinal secretions) showed a delay in labelling. Hair, fingernails, and bone were labelled more slowly.

These results can be expressed quantitatively by reference to a 3-compartment model (fig. 3) wherein compartment 1 represents the blood and those tissues which exchange rapidly ($\lesssim$ 3 days) with the blood. Introduction of compartment 2 permits representation of the delay in labelling found for the bile and other secretions, and

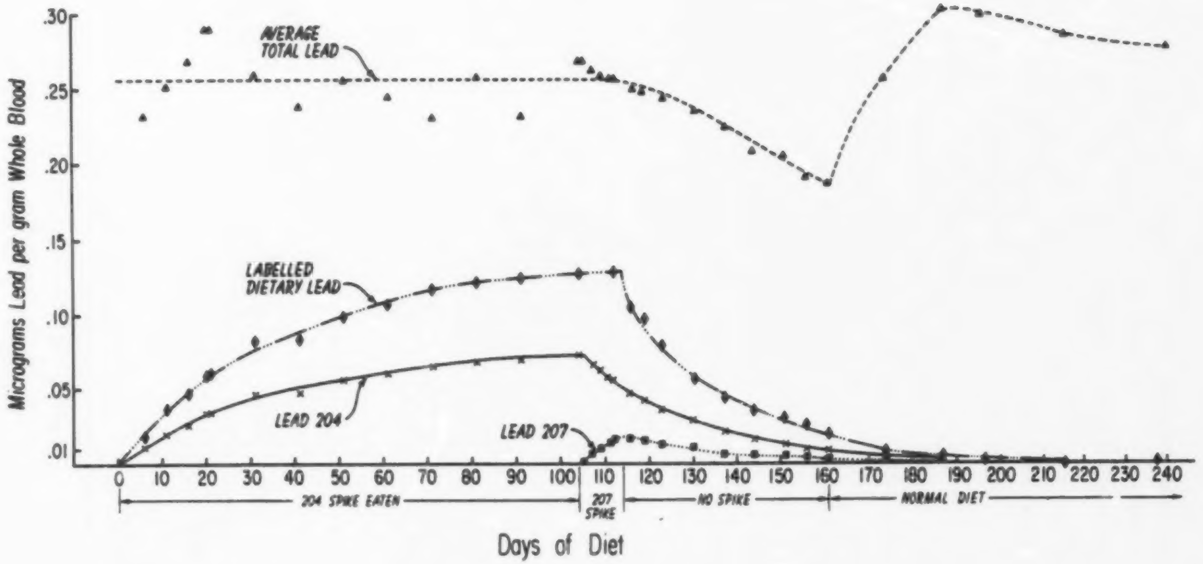

Figure 1. Blood lead concentration of Subject A. In the response to the diet containing labelled lead, the blood lead showed the gradual appearance of labelled lead. However, not all the blood lead will become labelled because of sources of unlabelled lead, such as the atmosphere and the skelton. When the dietary supplements of ^{204}PB and ^{207}PB are withdrawn, the total lead concentrations in the blood decreases by a corresponding amount. This indicates there is little tendency for the blood to maintain a constant lead concentration in this concentration range.

Blood Lead Concentration vs Time (Subject B)

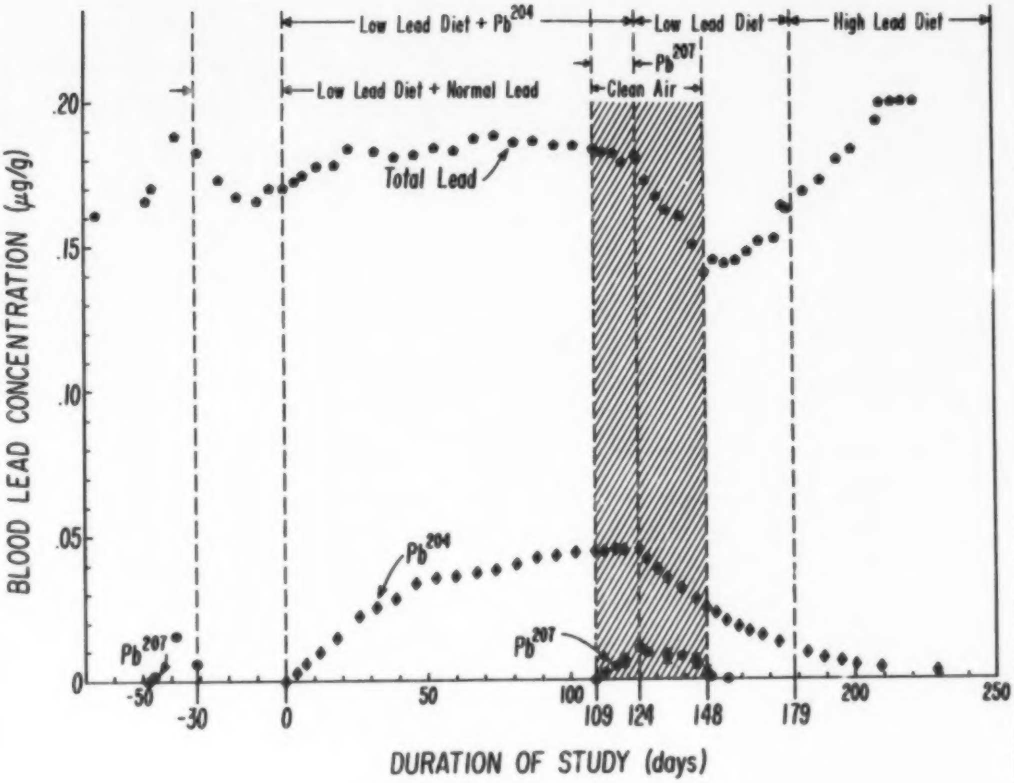

Figure 2. Blood lead concentration of Subject B. On day 109 a dietary supplement of ^{207}Pb was given in addition to the ^{204}Pb, and at the same time atmospheric lead was removed by filtering. The total lead dropped slightly, reflecting the fact that the excess ^{207}Pb did not exactly balance the loss of atmospheric lead. On day 124, the dietary supplements were discontinued. The only remaining lead source was the low lead diet. On day 148 the atmospheric lead was reintroduced, and the total lead started to rise. On day 179 the normal "high lead" diet was reintroduced. Detailed analysis of these results permits calculation of the atmospheric contribution given in table 3.

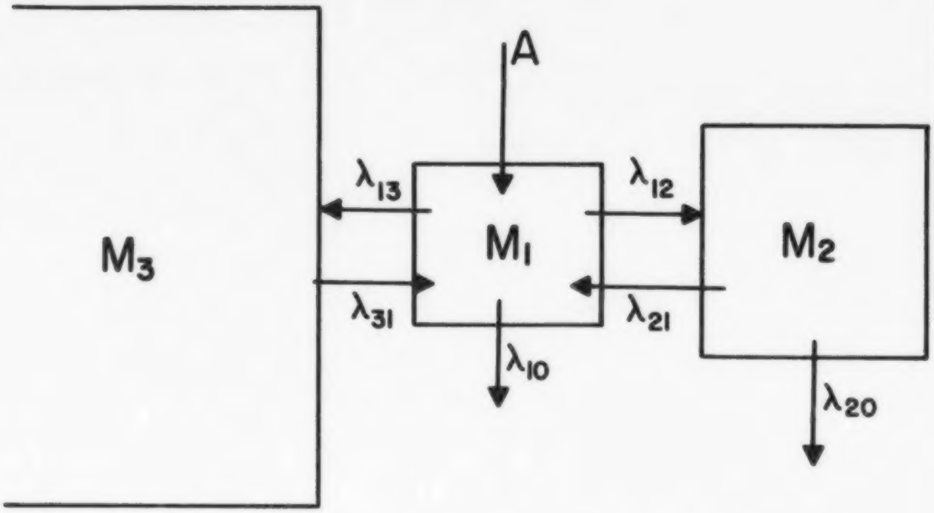

Figure 3. 3-compartment model used to obtain the parameters of table 1. M_1 is a short-lived compartment consisting of the blood and a similar mass of tissues and/or fluids which exchange rapidly with the blood. M_2 is a short-lived compartment, the labelling of which is delayed and is the source of lead in gastrointestinal secretions. M_3 is a long-lived compartment containing most of the lead in the body, primarily in the skeleton.

compartment 3 may be identified with the long-lived lead pool of the skeleton. Such a model is only a first approximation to the complexity of human physiology. In fact, our data cannot be fitted exactly to this model. Nevertheless this is a useful way of comparing the principal similiarities and differences between different subjects. If desired, most results of the work could be formulated in a less model dependent way. The results of fitting the experimental data for the two subjects are given in table 1. The parameters of the model were obtained at a constant value of total blood lead. It is found, however, that they are not strongly dependent on the value of total blood lead, and have been successfully used to predict the response of blood lead to decreases in lead input up to 70% for periods of times sufficiently long to cause the total blood lead concentration to decrease by 20%.

Table 1

Model parameters

	Subject A	Subject B
Initial slope	.0024 ± .0001	.00115 ± 10 μg/gr day
Daily tracer absorbed	17.5 ± .2	11.9 ± .2 μg/day
Compartment 1 mass	7.3 ± .3	9.9 ± .1 Kg
Lead in Compartment 1	1830 ± 75	1825 ± 50 μg
Lead in Compartment 2	260 ± 100	900 ± 100 μg
λ_1	.035 ± .002	.024 ± .002 1/days
λ_{10}	.020 ± .002	.015 ± .002 "
λ_{12}	.010 ± .003	.006 ± .002 "
λ_{13}	.005 ± .005	.003 ± .002 "
λ_2	.07 ± .02	.03 ± .01
λ_{21}	.02 ± .01	.0002 ± .0001
λ_{20}	.05 ± .02	.03 ± .01

This report is not sufficiently long to allow adequate discussion of all the data. However, some details will be given regarding two aspects of the study, the contribution of atmospheric lead and the gastrointestinal absorption of dietary lead.

2. Contribution of atmospheric lead

In these experiments it is possible to obtain a quantitative value for the daily quantity of lead absorbed from a typical urban atmosphere (2 μg Pb/m^3) in several ways:

(1) The over-all lead balance during the course of the experiment.

(2) The failure of the blood to become completely labelled. The ^{206}Pb/^{204}Pb ratio of the blood asympototically approaches a value distinctly lower than that of the food, indicating a non-dietary

contribution of lead of normal isotopic composition to the blood.

(3) Response of the blood lead concentration to removal and reintroduction of air lead by the use of atmospheric filters.

The first two methods are dependent on the assumption that transfer of lead from the skeleton to the blood is equal to the transfer of lead from the blood to the skeleton. Otherwise an excess transfer of unlabelled lead from the skeleton will mimic an atmospheric source of unlabelled lead. This assumption is avoided in the third method, used for the second Subject (B). Results of the three methods are shown in table 2. The good agreement found between the methods is evidence that the assumption of a steady state for exchange with the skeleton is valid, an assumption supported by other data. The value found, ~ 16 μg/day is somewhat less than quantity absorbed daily from typical diets (24 and 33 μg). The result is in good agreement with estimates based on the measured concentration of aerosol and vapor lead in the air breathed by the subjects, estimates of daily respired volume and lung absorption data, as measured by Booker et al [3] and Hursh and Mercer [4]

Table 2

Respired Lead Intake (μg/day)

Subject	Balance	Blood Labelling	Response to Filtered Air
A	16 ± 4	17 ± 5	-
B	19 ± 3	16 ± 6	16 ± 3

3. Absorption of dietary lead

During the two long-term (~ 6 month) studies, ^{204}Pb was substituted for approximately ½ the lead in the subject's pre-study diet. The ^{204}Pb was ingested as the nitrate in equal quantities with each meal. Under the controlled conditions of constant diet, environment, and daily routine, the absorption of ^{204}Pb by the gut, determined by difference between dietary and fecal lead, was quite constant (8.5% and 6.5% for Subjects A and B respectively). In contrast to the case of dietary lead of normal isotopic concentration, absence of

^{204}Pb in possible sources of contamination of the samples permitted rather accurate measurement of the absorption factor. These factors were different for the two subjects, but within the range reported by Hursh and Suomela [5].

As discussed in the previous section, even in this urban environment, the most important single source of absorbed lead was dietary. Furthermore the potentially available lead is much greater in the diet, since 40-50% of the total atmospheric lead is already being absorbed, in comparison with less than 10% of the dietary lead. In assessing the significant sources of lead in humans it is therefore necessary to study the variability of the gastrointestinal absorption when the controls on this quantity are relaxed.

This was done near the end of the long term study with Subject B. On two occasions the effect of fasting and chemical form were studied. In the first experiment, the subject fasted for 8 hours and then ate about 75 μg each of ^{204}Pb nitrate, ^{206}Pb cysteine, and ^{207}Pb sulfide and continued the fast for 6 more hours. This was repeated 35 days later with ^{204}Pb sulfide and ^{207}Pb nitrate. The next day ^{206}Pb cysteine was eaten with food. Absorption was measured in two ways: collecting and measuring unabsorbed lead in feces for 10 days following ingestion and measuring whole blood 2, 6 and 24 hours post-ingestion. Absorption could be determined from the blood data because the response of the blood lead to absorption had been well established during the long term study. The absorption factors found are shown in table 3. The results by the two methods may be considered to be in agreement considering they were based on a single day's response. The striking thing was the high absorption found while fasting. This greatly enhanced absorption (up to 50%, i.e. 8-fold) was found in both experiments, and must be considered a real effect.

In order to learn if this effect was peculiar to Subject B, a similar experiment was performed on another Subject (C). This subject had not participated in a long-term study and his blood response was not previously calibrated. This was done in a single measurement, using ^{206}Pb nitrate, taken with breakfast. This was compared with the fasting absorption of Pb nitrate and Pb sulfide The absorption found for ^{204}Pb nitrate with food (14%), was higher

Table 3

Effect of fasting and chemical form on lead absorption

Subject	Mode	Absorption (diet-feces) %	Absorption (blood response) %
B	fasting nitrate	40	35
B	" "	28	25
B	fasting sulfide	52	45
B	" "	45	41
B	fasting cysteine	30	22
B	cysteine with food	6	6
B	nitrate with food	6	
C	fasting nitrate	36	41
C	fasting sulfide	14	14
C	nitrate with food	14	
A	nitrate with food	8.5	

than previously found for the other two subjects. While fasting, much more lead nitrate was absorbed, whereas lead sulfide while fasting was only 14% absorbed.

These experiments show that gastrointestinal lead absorption varies between subjects, and is dependent upon chemical form. Most significantly, there is a pronounced tendency toward greater absorption when lead is ingested without food. This effect should be considered in connection with evaluation of absorption following ingestion of lead from paint, putty, etc. by children. The variability in absorption found in these experiments shows that the lead absorbed by a given individual is more than simply a function of the total lead ingested, and that much more information is required before the relationship between dietary and absorbed lead is understood.

These results were obtained by Veterans Administration Project No. 5016-02, and were supported by NSF RANN grant GI 38339.

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DISCUSSION

MAGI (Italy)

I should like confirmation of the theory that 75% of the blood lead is taken in with food whilst the remaining 25% is absorbed from the atmosphere.

Was an accumulation of Pb^{204} found in the blood and at what levels did this cease?

WETHERILL (U.S.A.)

Actually about 35% is absorbed from the atmosphere (when the concentration is around 2 µg Pb/m^3) and about 65% from food. Of course this will depend upon the lead content of the food eaten, on the quantity of food and the lead concentration of the atmosphere.

As shown in our table 2, about 16 ug of Pb was absorbed per day from the atmosphere. As explained in section 2 of the text, this was measured in 3 ways. One of these was based on the fact that the blood did not become entirely labelled (Fig. 1 and Fig.2

The Pb^{204} accumulated in the blood during the first part of the experiment and approached a steady state value of about .07 ug/g after about 100 days.

PFANNHAUSER (Austria)

In connection with the intake of lead from food, I should like to ask if the relatively high level was also found with aerosols, which are not metal lead but lead oxides?

WETHERILL (U.S.A.)

We do not have an accurate value for the absorption of Pb from aersols for two reasons:

1. The atmospheric lead was not isotopically labelled.
2. The respired volume during the entire course of the experiment can only be estimated.

Reason (2) is the more serious.

As discussed in section 2 of the text, the daily quantity of atmospheric lead absorbed (~16 ug/day) is in agreement with estimates of a typical daily respired volume and the approximate 40% Pb absorption found by other workers.

COFFIELD (Belgium)

What was the length of time subject B was held in the no lead atmosphere without changing any other experimental detail? Did the subject come to equilibrium with regard to blood lead level when there was no lead in air?

WETHERILL (U.S.A.)

The subject was maintained in the "clean air" environment for a total of 39 days. During the first 15 days, a sufficient quantity of Pb^{207} was added to this diet to offset the anticipate decrease in absorption of respired lead. This was very nearly achieved, the total lead in the blood only dropping slightly. Comparison of the increase in Pb in the blood with the decrease in concentration of Pb of normal isotopic composition (not shown in fig. 2) permits calculation of the quantity of respired lead removed without waiting to achieve a steady state. During the remaining 24 days, all dietary isotopic supplements (Pb^{204} and Pb^{207}) were removed, causing the total blood lead to decrease. The decrease in lead of normal isotopic composition caused by the removal of atmospheric lead continued as before, in agreement with our previous finding that homeostatic mechanisms do not operate to maintain a constant blood level. Upon reintroduction of air containing Pb and return to diet of previous lead content the blood lead concentrations increased as expected. The subject did not come to equilibrium with regard to blood lead level during this part of the experiment. However, this is not necessary. The non-equilibrium response can be measured just as well, without the considerable inconvenience to the subject which would result from maintaining the atmospheric control for > 100 days, as required to achieve a steady state. I would like to point out that this experiment was designed to measure the contribution of atmospheric lead at or near the subject's pre-study level of blood lead concentration. This does not necessarily or logically imply that the steady state blood lead concentration would be proportionately lower, following the removal of atmospheric lead. First of all, the contribution from deeper compartments, primarily the skeleton, would continue. We estimate this to be about 7 /ug/day, but the exact figure is uncertain.

Furthermore, it is possible that at sufficiently lowered blood lead concentrations homeostatic mechanisms may operate which tend to maintain a constant value of blood lead. We have found no evidence for such mechanisms when the total blood concentration is lowered by up to 25% but it is conceivable that at lower concentrations such an effect could occur.

BERLIN (C.E.C.)

In view of the attention presently given to atmospheric lead and of the fact that your subjects were maintained indoors in controlled atmospheres did you:

- sample regularly the indoor atmospheric lead concentration, and if yes with what method?
- determine the granulometry of the lead aerosols present in the ambiant indoor air?.

WETHERILL (U.S.A.)

Indoor atmospheric lead concentrations were continuously measured by capturing particulate lead on a millipore membrane filter (0.45 /u HAWP), with a retention rate > 95% for particles ≥ .05 /u. Air was pulled through this filter at a rate of approximately 1 m^3/hr. for 5 day collection periods. The lead on the filter was determined by stable isotope dilution. The sampler hung above the subject's bed. During the "clean air" phase of the study, the air lead levels in the subject's room were lowered to 0.072 ug/meter (0.062 to 0.087) from about 2 ug/m^3. No detailed examination of particle sizes was carried out. However, an activated carbon scrubber supplied by Dr. Ter Haar of Ethyl Corporation was placed behind the membrane filter to capture "vapor" lead. This captured an additional 0.10 ug/m^3 in outdoor air and 0.05 ug/m^3 in "clean air". We do not know the chemical or physical form of this "vapor" lead except that it was able to penetrate the membrane filter to be captured by the carbon. It could perhaps be TEL vapor or small (<.05 u) particles of lead salts.

SUL PASSAGGIO TRANSPLACENTARE DI INSETTICIDI CLORURATI

C. GRASSO

Istituto di Igiene dell'Università di Firenze, Italia

RIASSUNTO

Sono stati ricercati e titolati gli insetticidi clorurati presenti in campioni di sangue prelevati a puerpere e ai rispettivi figli neonati. I prelievi nei figli sono stati eseguiti prima che questi avessero assunto il primo pasto, naturale o artificiale, in maniera da titolare sicuramente soltanto gli insetticidi clorurati pervenuti al figlio dalla madre, escludendo totalmente l'apporto di tali sostanze nel periodo immediatamente successivo alla nascita.

Le titolazioni sono state eseguite nel sangue in quanto esso rappresenta la via di trasporto dagli insetticidi ai vari tessuti.

I campioni di sangue sono stati prelevati nella clinica Ostetrica dell'Università di Firenze e nel reparto Pediatrico dell'Ospedale di Arezzo, da donne di varia estrazione sociale, lavoro, domicilio, e dai rispettivi figli, alcuni nati a termine e sani (Firenze) e altri prematuri o affetti da qualche forma patologica (Arezzo). Complessivamente sono stati esaminati 70 campioni di sangue. Gli insetticidi sono stati ricercati gascromatograficamente, con la seguente metodica: - estrazione degli insetticidi dal sangue con solventi; - purificazione dell'eluato in colonna di allumina; - analisi gascromatografica con detector a cattura di elettroni (Stronzio 90). Tutti i nostri cromatogrammi hanno presentato picchi di insetticidi clorurati. In tutti i campioni esaminati è presente il pp'DDT in quantità che arrivano oltre le 0,6ppm, il DDD e il DDE. Gli

altri insetticidi ricercati (lindano, eptacloro, eptacloro epossido, aldrin, dieldrin) sono presenti nella maggior parte dei campioni con eccezione dell'eptacloro epossido e del dieldrin, presenti rispettivamente solo in 4 e in 9 campioni.

I cromatogrammi degli insetticidi rilevati nel sangue delle madri e dei rispettivi figli sono simili qualitativamente, ma non quantitativamente, essendo sempre superiori i tassi reperiti nel sangue della madre rispetto a quelli dei figli.

Risulta dunque che almeno in parte gli insetticidi presenti nel sangue della madre passano durante la gravidanza nel sangue del figlio.

Pressocchè per tutti gli insetticidi, e tanto nel gruppo delle madri che in quello dei figli, i tassi medi riscontrati sono risultati superiori nei soggetti di Arezzo rispetto a quelli di Firenze. Sono in corso altre prove per valutare l'eventuale correlazione di questo reperto con lo stato di salute dei neonati.

ABSTRACT

The chlorinated insecticides present in samples of blood taken from puerperae and their children were investigated and identified. The samples were taken before the infants had been given their first breast or bottle feed, so as to be sure of titrating only those chlorinated insecticides which had reached the child from the mother, by totally excluding the introduction of these substances in the period immediately following birth.

The titrations were carried out on the blood, since this is the vehicle by which the insecticides reach the various tissues.

The blood samples were taken at the Obstetrical Clinic of the University of Florence and in the pediatrics department of the Arezzo Hospital from women of various social categories, working women and housewives, and from their offspring, some being healthy children born at term, (Florence) and others born prematurely or with some pathological condition (Arezzo). 70 blood samples were examined in all.

The insecticides were examined by gas chromatography, the technique being as follows: - the insecticides were solvent-extracted from the blood, the eluate was purified in an alumina column, and a gas chromatography analysis was made with an electronic capture detector (Strontium 90). All our chromatograms showed peaks of chlorinated insecticides. In all the samples pp'DDT was present in quantities exceeding 0.6 ppm and also DDD and DDE. The other insecticides investigated (lindane, heptachlorine, heptachlorine epoxide, aldrin and dieldrin) were present in most of the samples with the exception of heptachlorine epoxide and dieldrin, which were found only in 4 and 9 samples respectively.

The chromatograms of the insecticides taken from the blood of the mothers and their children were similar in quality, but not in quantity, the counts found in the mothers' blood being in each case higher than those for the children.

This shows that to some extent at least the insecticides in the mother's blood pass into the child's blood during pregnancy.

The average count of nearly all the insecticides, in the mothers as well as in the children, were higher for the subjects from Arezzo than for those from Florence. Further tests are in hand to see whether these results can be correlated with the state of health of the newborn children.

1. INTRODUZIONE

L'ormai accertata diffusissima presenza degli insetticidi clorurati nell'ambiente (Grasso [1-2]), e conseguentemente anche in tutti gli alimenti utilizzati dall'uomo (Grasso e coll., Mazzetti e coll. [3-4-5-6-7]), ha portato a ricercare e titolare le quantità di tali insetticidi che l'uomo adulto accumula nel proprio organismo durante la propria vita (Grasso e coll. [8]). Si dispone ormai di numerosi dati largamente sufficienti a dimostrare che queste sostanze si depositano in quantità apprezzabili nell'organismo umano, soprattutto nel tessuto adiposo.

Si era supposto da tempo che una certa quantità di insetticidi clorurati potesse anche passare dalla madre al figlio durante il periodo della gravidanza e fosse percio' reperibile nei tessuti del neonato. Questa ipotesi è stata controllata sia sugli animali da esperimento sia direttamente sull'uomo, anche con lo scopo di indagare sugli eventuali effetti nocivi che queste sostanze potrebbero produrre in tali circostanze.

Il passaggio placentare degli insetticidi clorurati fu dapprima dimostrato su cani, conigli, topi (Finnegan e coll., Backstrom e coll., Mohn e coll. [9-10-11]). Successivamente furono eseguite ricerche anche sull'uomo: furono analizzati tessuti di neonati di pochi mesi (Fiserova Bergerova e coll., Abbot e coll., Casarett e coll. [12-13-14]) ed in essi furono reperiti insetticidi clorurati, a conferma di quanto osservato negli animali.

In seguito furono condotte ricerche più accurate su neonati morti subito dopo la nascita, confrontando i titoli di insetticidi clorurati in essi rinvenuti con quelli dimostrati nella popolazione adulta vivente nello stesso ambiente e, in alcuni casi, nelle rispettive madri. Zavon e coll. [15], Gravibesen e coll. [16], Charley e coll. [17] seguendo questo indirizzo esaminarono i più diversi organi e tessuti tra cui il sangue del cordone ombelicale. Selby e coll. [18] ricercarono gli insetticidi clorurati tanto nel sangue materno quanto nel tessuto placentare delle stesse donne.

I risultati di tutte le suddette ricerche concordano nell'indicare la possibilità del passaggio placentare di tutti gli insetticidi clorurati, dimostrando la presenza nei neonati di tali sostanze in quantità eguali o di poco inferiori a quelle riscontrate nella popolazione adulta della zona o addirittura nelle rispettive madri.

La nostra ricerca, traendo spunto dalle precedenti, si proponeva di indagare nella maniera più precisa e più attendibile possibile il passaggio degli insetticidi clorurati dalle madri ai rispettivi figli durante la gravidanza. A questo scopo si è proceduto alla titolazione di tali sostanze sia nel sangue prelevato dalle puerpere che in quello prelevato dai rispettivi figli, sani o portatori di una qualsiasi patologia, prima che essi avessero assunto il primo pasto, naturale o artificiale che fosse.

La metodica adottata, al contrario di quelle utilizzate nella maggior parte delle ricerche precedenti, consente di titolare sicuramente soltanto gli insetticidi clorurati pervenuti al figlio dalla madre, escludendo totalmente l'influenza di qualunque apporto di tali sostanze nel periodo successivo alla nascita.

2. MATERIALE E METODO

Per eseguire le titolazioni programmate secondo i criteri sopra esposti, è stato scelto come materiale da esaminare il sangue, sia in base a considerazioni pratiche relative alla semplicità del prelievo, trattandosi di bambini vivi, sia in base a considerazioni relative al fatto che gli insetticidi clorurati pervenuti nell'organismo, usufruiscono del sangue come via di trasporto ai tessuti.

I campioni di sangue sono stati prelevati nelle provincie di Arezzo e di Firenze, da donne di varia estrazione sociale, domicilio, lavoro, comunque tutte presumibilmente esposte allo stesso rischio di assunzione di insetticidi clorurati, e dai rispettivi figli, dei quali alcuni nati a termine ed altri prematuri. Tutti i neonati di Arezzo erano affetti da qualche forma patologica (ittero, broncopolmonite, emorragia intracranica), mentre tutti quelli di Firenze erano sani al momento della nascita.

I campioni di sangue sono stati prelevati presso la Clinica Ostetrica e Ginecologica dell'Università di Firenze e presso il Reparto di Pediatria dell'Ospedale di Arezzo, ove i Primari e tutto il personale, medico e non medico, ci hanno fornito una preziosa collaborazione.

La titolazione degli insetticidi clorurati è stata eseguita per mezzo di gas-cromatografia con detector a cattura di elettroni.

Sono stati ricercati e titolati i seguenti insetticidi: pp'DDT, DDE, DDD, Lindano, Eptacloro, Eptacloro Epossido, Aldrin, Dieldrin.

Il metodo utilizzato per l'analisi gas-cromatografica è il seguente. I campioni di sangue sono stati trattati con EDTA per evitarne la coagulazione; ml 1 del campione è stato messo in un imbuto separatore da ml 10, ad esso sono stati aggiunti ml 3 di una miscela acetone-etere etilico 1:1, si è agitato il tutto per un minuto e si sono lasciate separate le due fasi; si è poi ripresa la fase etere-acetone e tale operazione è stata ripetuta tre volte. Gli estratti sono stati riuniti e messi ad evaporare fino a completo essiccamento; il residuo è stato ripreso con ml 2 di benzolo, passato in colonna di allumina ed eluito con ml 75 di benzolo. L'eluato, evaporato fino a ml 1, è stato iniettato nel gas-cromatografo con siringa Hamilton in quantità tali da poter evidenziare gli insetticidi presenti. Il gas-cromatografo usato è un RSCo con detector a cattura di elettroni (stronzio 90), con colonna di vetro (lunghezza m. 1,90, diametro interno mm. 4, fase stazionaria QF^1 15% su DC 200) e con registratore Honeywell Electronik 0,1 mV. Le temperature di esercizio sono le seguenti: iniettore 220°C, colonna 210°C, detector 220°C. Gas di trasporto: azoto PP; flusso 120 ml/min.

Solventi usati: acetone, etere etilico, benzolo per spettrofotometria ridistillati.

Tutti i nostri cromatogrammi hanno presentato picchi di insetticidi clorurati.

In precedenza avevamo fatte soluzioni standards dei diversi principi attivi puri al 99,9% in benzolo puro per spettrografia, ridistillato. Dalla soluzione 1.10^{-2} g/ml, eseguita per pesata, si perveniva, per successive diluizioni, alle concentrazioni 1.10^{-6} e 1.10^{-7} g/l. Di queste soluzioni è stata fatta una serie di iniezioni con siringa Hamilton da 1 a 10 µl e se ne è misurata l'altezza in mm. dei corrispondenti picchi.

In un grafico in cui in ascisse sono riportate le quantità iniettate in µl e in ordinate l'altezza dei picchi dei cromatogrammi dei campioni di sangue con quelli delle soluzioni standards è applicata la formula proposta da Hartman per la determinazione quantitativa:

$$\text{concentrazione in ppm} = \frac{V\ w\ h2}{W\ v\ h1}$$

in cui V = volume estratto in ml
w = peso della soluzione standard in ng
v = volume dell'estratto iniettato in ml
W = peso del campione in g
h1 = altezza in mm del picco della soluzione standard
h2 = altezza in mm del picco del sangue

3. RISULTATI

I risultati delle titolazioni eseguite sono riportati in tab. 1. I dati qui esaminati si riferiscono a campioni di sangue prelevati da 21 neonati fiorentini privi di manifestazioni patologiche e dalle rispettive madri; e inoltre da 14 neonati aretini portatori di qualche manifestazione patologica e dalle rispettive madri. Si tratta quindi, complessivamente, di 70 campioni di sangue.

L'osservazione dei dati riportati in tab. 1 consente le seguenti considerazioni principali:

1. In tutti i 70 campioni di sangue esaminati è presente il pp'DDT in quantità relativamente elevate, fino ad oltre 0,6 ppm; anche il DDD e il DDE sono presenti in tutti i campioni, per quanto in ciascun caso in quantità notevolmente inferiori a quelli del pp'DDT, ma senza consistenti differenze tra di loro.
2. Degli altri insetticidi clorurati ricercati, il Lindano, l'Eptacloro e l'Aldrin sono presenti nella maggioranza dei campioni di sangue, con frequenza e con concentrazioni decrescenti nell'ordine sopra indicato; l'Eptacloro Epossido e il Dieldrin, metaboliti rispettivamente dell' Eptacloro e dell'-Aldrin, sono al contrario presenti solo in un numero estremamente esiguo di campioni (rispettivamente 1 e 9).
3. I risultati appaiono sostanzialmente simili nel gruppo delle madri e in quello dei neonati. Per quanto nella maggioranza dei casi tanto i valori singoli che quelli medi riscontrati nelle madri (tab. 2) siano di poco superiori a quelli dei figli, tali differenze dimostrano infatti ben di rado una significatività statistica (tab. 3): soltanto in un caso

TABELLA 1.

Tassi di insetticidi clorurati riscontrati su campioni di sangue di madri e di rispettivi neonati delle provincie di Arezzo e Firenze.

Provenienza			Categoria			Tassi ematici ppm 10^{-5}							
				Figli									
	FI	AR	Madri	non Pat.	Pat.	pp'DDT	DDE	DDD	Lindano	Epta-cloro	Eptacloro Ep.	Aldrin	Dieldrin
1	X		X			3.470	611	343	121	1.230	-	tracce	-
	X			X		3.810	515	750	113	1.160	-	tracce	-
2	X		X			10.000	109	850	246	597	-	tracce	-
	X			X		3.300	513	312	-	119	835	tracce	-
3	X		X			6.110	115	375	779	652	-	tracce	-
	X			X		6.650	763	550	974	583	-	tracce	-
4	X		X			3.290	398	449	168	714	-	tracce	-
	X			X		7.410	773	899	196	574	-	tracce	-
5	X		X			4.420	954	500	137	510	-	tracce	-
	X			X		9.610	619	608	784	838	-		-

TABELLA 1. (Seguito)

	Provenienza		Categoria			Tassi ematici ppm 10^{-5}							
				Figli									
	FI	AR	Madri	non Pat.	Pat.	pp'DDT	DDE	DDD	Lindano	Epta-cloro	Eptacloro Ep.	Aldrin	Dieldrin
6	X		X			5.000	716	388	1.220	1.080	-	tracce	-
	X			X		6.300	482	515	459	691	-	tracce	-
7	X		X			6.530	750	611	186	652	-	tracce	-
	X			X		6.300	438	343	156	521	-	tracce	-
8	X		X			1.500	839	662	1.750	1.500	-	119	-
	X			X		4.400	312	272	2.300	892	942	85	-
9	X		X			4.800	575	391	6.920	550	-	71	-
	X			X		4.400	475	501	480	450	-	450	-
10	X		X			1.600	409	200	1.410	-	-	28	-
	X			X		1.600	287	280	162	-	-	26	-
11	X		X			2.330	359	266	528	804	-	73	-
	X			X		4.000	196	280	337	-	-	42	-

TABELLA 1. (Seguito)

	Provenienza		Categoria			Tassi ematici ppm 10^{-5}							
				Figli									
	FI	AR	Madri	non Pat.	Pat.	pp'DDT	DDE	DDD	Lindano	Epta-cloro	Eptacloro Ep.	Aldrin	Dieldrin
12	X		X			2.500	441	366	4.660	687	-	773	-
	X			X		1.830	233	233	112	-	-	-	-
13	X		X			2.000	315	433	450	-	-	71	-
	X			X		1.830	378	400	574	-	-	140	-
14	X		X			847	227	266	1.080	1.310	-	99	-
	X			X		847	227	266	1.820	847	-	132	-
15	X		X			1.700	400	235	297	709	-	425	-
	X			X		1.000	183	205	106	554	-	300	-
16	X		X			2.450	406	324	894	1.560	-	206	-
	X			X		2.350	362	388	142	550	-	47	-
17	X		X			2.000	306	272	257	-	-	-	-
	X			X		7.500	125	181	138	-	-	-	-

TABELLA 1. (Seguito)

	Provenienza		Categoria			Tassi ematici ppm 10^{-5}							
				Figli									
	FI	AR	Madri	non Pat.	Pat.	pp'DDT	DDE	DDD	Lindano	Epta-cloro	Eptacloro Ep.	Aldrin	Dieldrin
18	X		X			1.000	312	227	185	787	-	418	-
	X			X		2.330	375	357	608	206	-	134	-
19	X		X			1.660	566	250	425	1.510	-	115	-
	X			X		2.500	383	285	398	1.450	-	862	-
20	X		X			2.900	375	400	659	1.310	-	67	-
	X			X		3.540	312	300	411	800	-	48	-
21	X		X			2.940	541	231	381	854	-	595	657
	X			X		13.900	879	833	165	449	-	-	-
22		X	X			44.300	4.840	1.510	720	1.650	-	120	-
		X			X	16.400	3.860	333	1.000	630	-	210	-
23		X	X			30.100	3.230	616	930	4.520	-	240	-
		X			X	61.900	4.760	2.840	312	870	-	87	-

TABELLA 1. (Seguito)

Provenienza			Categoria			Tassi ematici ppm 10^{-5}							
				Figli									
	FI	AR	Madri	non Pat.	Pat.	pp'DDT	DDE	DDD	Lindano	Eptacloro	Eptacloro Ep.	Aldrin	Dieldrin
24		X	X			11.100	1.300	857	8.580	1.940	-	122	348
		X			X	7.500	1.080	840	650	1.000	-	140	208
25		X	X			7.140	866	571	4.700	6.220	-	122	370
		X			X	4.400	1.540	400	780	1.100	-	100	375
26		X	X			3.170	392	347	418	600	-	-	-
		X			X	9.440	730	400	675	893	-	tracce	-
27		X	X			3.330	459	282	156	372	-	50	-
		X			X	8.330	3.490	923	160	3.870	-	370	-
28		X	X			2.820	439	391	4.600	647	-	tracce	-
		X			X	7.770	3.120	300	5.260	175	-	tracce	-
29		X	X			3.900	609	400	3.710	941	-	71	294
		X			X	9.760	2.880	789	375	675	-	362	-

TABELLA 1. (Seguito)

Provenienza			Categoria			Tassi ematici ppm 10^{-5}							
				Figli									
	FI	AR	Madri	non Pat.	Pat.	pp'DDT	DDE	DDD	Lindano	Eptacloro	Eptacloro Ep.	Aldrin	Dieldrin
30		X	X			36.600	4.500	760	2.280	3.650	-	-	-
		X			X	34.700	5.660	6.250	2.590	3.110	-	203	-
31		X	X			30.300	4.070	2.400	7.690	3.510	-	tracce	-
		X			X	7.070	5.160	125	2.080	2.830	-	176	250
32		X	X			46.000	4.600	5.010	2.030	2.960	-	tracce	-
		X			X	31.000	4.170	710	1.160	1.500	1.500	2.300	280
33		X	X			8.130	714	500	592	800	-	tracce	-
		X			X	11.300	656	454	141	200	-	tracce	-
34		X	X			12.600	1.710	1.770	1.270	4.090	1.010	530	2.150
		X			X	7.540	2.660	280	2.320	371	-	223	-
35		X	X			5.710	452	400	192	404	-	-	-
		X			X	20.750	1.490	710	96	120	-	tracce	-

TABELLA 2

Valori medi relativi ai tassi dei singoli insetticidi clorurati riscontrati in campioni di sangue di madri e dei rispettivi neonati delle provincie di Arezzo e Firenze

Insetticida	Valori medi su ppm 10^{-5}			
	Campioni di Firenze(n.21)		Campioni di Arezzo (n.14)	
	Madri	Neonati	Madri	Neonati
pp'DDT	3.287±2.210	4.543±3.960	17.514±16.220	17.030±15.900
DDE	463± 222	420± 204	2.012± 1.800	2.946± 1.660
DDD	382± 163	417± 206	1.129± 1.281	1.096± 1.622
LINDANO	1.083±1.665	496± 581	2.704± 2.778	1.252± 1.388
EPTACLORO	810± 376	508± 409	2.307± 1.792	1.239± 1.185
EPTACLORO EP.	-	84± 267	72± 270	79± 390
ALDRIN	145± 219	107± 207	89± 146	262± 589
DIELDRIN	31± 143	-	225± 571	79± 137

TABELLA 3

Significatività statistica delle differenze dei valori medi di insetticidi clorurati riscontrati su campioni di sangue di madri e dei rispettivi neonati delle provincie di Arezzo e Firenze

Insetticida	Firenze Madri | Figli	Arezzo Madri | Figli	Neonati Arezzo | Firenze	Madri Arezzo | Firenze
pp'DDT	$0{,}02 > t > 0{,}01$	$0{,}90 \geq t > 0{,}80$	$0{,}02 > t > 0{,}01$	$0{,}001 \geq t$
DDE	$0{,}60 > t > 0{,}50$	$0{,}02 > t \geq 0{,}01$	$0{,}001 > t$	$0{,}02 > t > 0{,}01$
DDD	$0{,}60 > t > 0{,}50$	$0{,}95 > t > 0{,}90$	$0{,}10 > t > 0{,}05$	$0{,}10 > t > 0{,}05$
LINDANO	$0{,}20 > t > 0{,}10$	$0{,}02 > t > 0{,}01$	$0{,}20 > t > 0{,}10$	$0{,}20 > t > 0{,}10$
EPTACLORO	$0{,}001 > t$	$0{,}02 > t > 0{,}01$	$0{,}20 > t > 0{,}10$	$0{,}05 > t > 0{,}025$
EPTACLORO EP.	$0{,}30 > t > 0{,}25$	$0{,}90 > t > 0{,}80$	$0{,}90 > t > 0{,}80$	$0{,}30 > t > 0{,}25$
ALDRIN	$0{,}60 > t > 0{,}50$	$0{,}25 > t > 0{,}20$	$0{,}001 > t$	$0{,}50 > t > 0{,}40$
DIELDRIN	$0{,}60 > t > 0{,}50$	$0{,}40 > t > 0{,}30$	$0{,}30 > t \geq 0{,}20$	$0{,}20 > t > 0{,}10$

(coppie di Firenze, Eptacloro) la significatività statistica supera il valore di $t = 0,001$; in quattro casi poi (coppie di Firenze: pp'DDT; coppie di Arezzo: Lindano, Eptacloro e DDE) il valore di 7 è compreso fra 0,02 e 0,01.

4. Pressochè per tutti gli insetticidi, e tanto nel gruppo delle madri che in quello dei figli, i tassi medi riscontrati sono risultati superiori nei soggetti di Arezzo che in quelli di Firenze (tab. 2); tuttavia una concreta significatività statistica della differenza dei valori medi ($t < 0.001$) ha potuto essere evidenziata, per quanto riguarda i neonati, solo per il DDE e l'Aldrin; per quanto, invece, riguarda le madri il valore di t è il risultato inferiore a 0.001 soltanto per il pp'DDT (tab. 3).

5. I neonati aretini presentano i quadri patologici più vari e non è pertanto possibile, dato il basso numero di osservazioni, tentare di correlare i tassi ematici degli insetticidi clorurati con le manifestazioni morbose dei neionati. Anche la ricerca di un eventuale rapporto tra tasso ematico di insetticidi clorurati e peso alla nascita (ipotesi giustificata da talune osservazioni sperimentali relative all'azione di tali sostanze sul metabolismo del Ca e quindi presumibilmente nel processo di ossificazione) non conduce ad alcun risultato concreto per analoghi motivi.

In conclusione, le ricerche in questa sede riferite dimostrano con sicurezza la costante presenza di insetticidi clorurati nel sangue di neonati prima dell'assunzione del primo pasto e sembrano pertanto convalidare senza riserve, data la sicura esclusione di un'origine alimentare post-natale dei suddetti insetticidi, la possibilità del passaggio per via placentare di tali sostanze, che in effetti si trovano costantemente anche nel sangue materno.

Il reperimento di tassi ematici più elevati di insetticidi clorurati in neonati portatori di qualche manifestazione patologica e generati da madri esse pure presentanti tassi di tali sostanze relativamente maggiori lascia aperta l'ipotesi di una eventuale correlazione fra tali eventi (elevato livello ematico materno → elevato livello ematico neonatale → presenza di patologia neonatale).

Tuttavia il fatto che cio' si sia verificato esclusivamente nei campioni di sangue provenienti da una sola delle due circoscrizioni territoriali prese in esame lascia adito a talune perplessità. L'eventuale esistenza di una tale correlazione richiede quindi un più approfondito controllo, sulla base dell'analisi di un maggior numero di campioni, di un più chiaro riferimento a talune situazioni patologiche neonatali e di una più estesa e più precisa distribuzione topografica dei campioni prelevati.

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DISCUSSIONE

ROSIVAL (Cecoslovacchia)

Dai risultati delle nostre ricerche (Rosival, Szokolay, Uhnčk) emerge una prevalenza del DDT totale sul BHC totale a livello dell'utero nel secondo e terzo mese di gravidanza, ed inoltre una concentrazione di DDT totale e di isomeri β e γ di BHC più elevata inregioni pianeggianti che in regioni di media montagna. Il tasso del DDT é più elevato di quello del DDE (il livello medio del DDT in regioni pianeggianti é di 0,128 ppm; in regioni di media-montagna é di 0,028 ppm; il livello medio di DDE in regioni pianeggianti é di 0,046 ppm, in regioni di media-montagna di 0,006 ppm).

Cio' denota una diversità nella capacità di penetrazione dei residui nel caso del latte materno in rapporto alla costanza della correlazione fra DDT e DDE nel grasso umano, ed alla possibilità che la placenta svolga una funzione d'organo metabolico attivo.

La prevalenza del DDT sul DDE, indica sia breve durata all' esposizione che materiali a basso tenore in lipidi.

WASSERMAN (Israele)

I composti OCI e PCB attraversano la placenta e sul piano qualitativo li troviamo nei tessuti del neonato come pure nelle varie fasi della vita intrauterina. La più elevata esposizione all'OCI e PCB si verifica quando il neonato viene alimentato unicamente con latte materno, poiché quest'ultimo contiene aliquote più elevate di composti OCC che non il latte vaccino.
I dati forniti dalla D.ssa Grasso meritanto attenzione vista l'importanza di considerare le donne incinta come indice dell' esposizione tossica ambientale.

DANIEL (Gran Bretagna)

Ha la D.ssa Grasso costruito altri parametri per spiegare le osservazioni relative ai neonati di Arezzo?

Erano le donne dei due gruppi della medesima condizione sociale? Avevano esse il medesimo tenore alimentare? Nei gruppi considerati vi erano delle fumatrici?

E' forse prematuro attribuire gli effetti unicamente ai livelli di insetticidi clorurati presenti nel sangue materno.

GRASSO (Italia)

Le condizioni delle donne dei due gruppi erano le piu' simili possibili.

Non ho attribuito gli effetti agli insetticidi clorurati reperiti: ho detto che gli studi proseguono per approfondire l'osservazione fatta.

MODIFICATION OF THE HOMOLOG AND ISOMER COMPOSITION OF A POLYCHLORINATED BIPHENYL MIXTURE DURING PASSAGE THROUGH TWO BIOLOGICAL SYSTEMS

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ABSTRACT

The polychlorinated biphenyl (PCB) mixture Aroclor 1254 was administered to breeding populations of hens and rats at 6 mg/kg body weight for 6 weeks. The populations were observed then and during the subsequent 20-week clearance period. Egg yolks and tissues of adults, embryos and fetuses, chicks and pups were analyzed; and the fate of 16 individual homologs and isomers of Aroclor 1254 was observed during the build-up and decline periods. The toxicity of the PCB mixture in the hen can be attributed either to a metabolite of 3,4,2',3',6'-pentachlorobiphenyl or to the intrinsic toxicity of the more persistent 3,4,2',4',5'-pentachlorobiphenyl or 2,3,4,2',4',5'-hexachlorobiphenyl. In the rat, however, both of these pentachlorobiphenyls were eliminated relatively rapidly. Hence 4,4'- substitution is more important in determining persistence in the chicken than in the rat.

Analyses of rat fetuses showed that there was little placental transfer of PCB. However, PCB was passed from mothers to pups as soon as suckling began. By contrast, the chick embryo was exposed to PCB present in egg yolk from its earliest development.

Analyses of geese and duck collected in New York State confirmed results of laboratory studies with regard to PCB persistence. The laboratory results indicate that the concentrations of PCB in eggs of some wild birds, which as reported by other workers are frequently greater than 10 μg/g, may quite possibly be a factor in the reduced reproductive success of these species.

1. Introduction

The toxicity of commercially produced polychlorinated biphenyl (PCB) mixtures has been fairly well demonstrated and reviewed in recent years (e.g., Fishbein [1], Hammond et al. [2]). The toxicity of the mixtures themselves and of several components of typical mixtures has been determined in a wide variety of living organisms. In long-term low-level experiments, the concentrations of PCB in various tissues of populations of animals have been determined. In short-term experiments, metabolic products of several individual PCBs have been isolated and characterized. In some studies with commercial mixtures, metabolic changes in composition of the ingested mixture have been noted; both changes with time and differences between organs in the same animal have been observed. A more thorough investigation of such changes was one of the primary aims of the present work.

The toxicity of PCB mixtures first drew wide attention in the poultry industry because newly hatched chicks are highly susceptible to the compounds. Since this rediscovery (the toxicity had been clearly demonstrated in 1937 by the U.S. Surgeon General [See 2]), residues of PCB found in wildlife have been blamed for reduction in the reproductive success of various species of birds and of some carnivorous mammals, e.g., otters and mink. Another aim of the present work, therefore, was to investigate the effect of a low-level intake of a common PCB mixture (Aroclor 1254) on the young of an avian and a mammalian species.

2. Experimental

Aroclor 1254, kindly donated by Monsanto Chemicals, St. Louis, Mo., was dissolved in the detergent Tween 80 (Sigma Chemical Co., St. Louis, Mo.) and then emulsified with water. The concentration was adjusted to give the animals a daily intake of 6 mg/kg body weight.

Twelve white Leghorn hens and two roosters were used for the first experiment. Details of the experiment and some conclusions have been reported by Tumasonis et al. [3] and Bush et al. [4] . Wistar rats (125 male, 125 female) were used in the second experiment.

Extraction of PCB from tissue samples and from eggs was carried out by the usual methods (e.g., Bush and Lo [5]). Possible residues of DDT-related insecticides derived from animal feed were eliminated by oxidative clean-up with chromic acid in acetic acid.

Analysis was carried out with 7600A Chromatographic System (Hewlett-Packard, Avondale, Pa.) using electron capture detection. The stationary phase was Apiezon L (2% on Gaschrom Q, 80-100 mesh), and the areas of the eighteen peaks, as identified by Sissons and Welti [6,7], were integrated. For quantitation, the area of each peak was divided by the area of the corresponding peak in the chromatogram produced by a known mass of Aroclor 1254. This ratio permitted a rough quantitation of the peak in μg/g. We have called each such value the notional concentration of the PCB component in the tissue extract. The mean of these notional concentrations gives an estimate of the total mass of PCB present in the tissue. The notional concentrations and their means were displayed as logarithmic bar charts using the Wang Programmable Calculator and Data Plotter (Wang Inc., Tewksbury, Mass.). An unmodified residue would give a set of 19 equal bars (18 peaks plus the mean). The value of each mean is printed above it.

3. Results and Discussion

3.1 The hen experiment

Embryonic mortality reached 100% in eggs laid by hens which had been exposed to the PCB intake for 3 weeks. Exposure was terminated 6 weeks after the commencement of the experiment. Chicks started to hatch again from eggs laid in the 15th week of the experiment, i.e., 9 weeks after termination of intake.

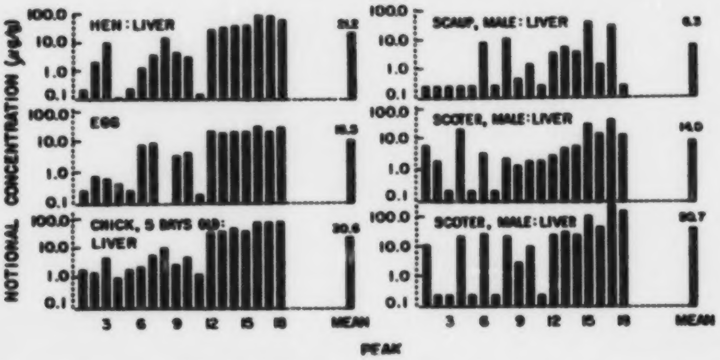


Figure 1. Aroclor 1254 modified by the White Leghorn nine weeks after termination of intake. PCB residues from contaminated New York State waterfowl.

Figure 1 shows the isomer and homolog pattern of residues recovered from a hen, an egg and a surviving chick, all derived from the 16th week of the experiment. It is clear that residence of the PCB mixture in the hens has produced a considerable change in the relative concentrations of the components. Moreover, the PCB deposited in the egg by the hen is incorporated into the chick during its embryonic development. We have earlier shown (Bush et al. [4]) that at this period of the experiment, the residual mixture was up to five times more toxic to embryos than the original mixture and that the increase in toxicity was most probably due to the relatively high concentrations of the components of peaks 12, 13 and 14. The patterns shown in Figure 1 can be used to indicate the potency of residues found in specimens with unknown histories, such as avian wildlife.

We showed previously (Bush et al. [4]) that of the four major constituents of Aroclor 1254, peak 9 (3,4,2',3',6'-pentachlorobiphenyl) did not persist in the hen system, whereas peaks 12, 13 and 14 did persist. After termination of intake, peak 9 soon declined until, at the stage shown in Figure 1, it was reduced ten times relative to peak 12. The ratio of these two peaks might therefore be used as a rough indication of the age of a residue. Figure 2 shows the variation of this ratio (12:9) in eggs laid during the course of the experiment. Despite the large dispersion, a trend is clear. So long as the ratio is below 10, it is probable that such a residue found in an egg or in the liver of a hen or chick is derived from an exposure terminated not more than 4 or 5 months earlier (liver composition and egg composition are highly correlated, Bush et al. [4]). Moreover, such a residue would be more toxic to embryos than an older residue containing relatively more of the hexa- and heptachlorobiphenyls.

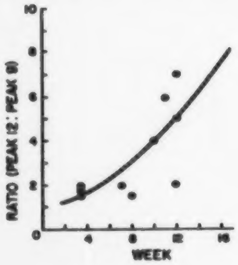


Figure 2. Change in the ratio of the notional concentration of peaks 12:9 in egg yolks with time.

Extrapolation to avian wildlife in general to indicate the absolute potency of a particular residue burden would not be valid. The 12:9 ratio might, however, provide a valuable indication of the age of a residue as one parameter when interpreting the data from surveys of wildlife populations.

3.2 Wildlife survey

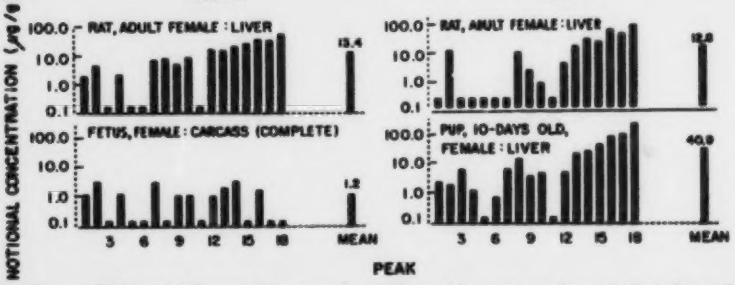
In a limited survey of avian wildlife in New York State, two species of duck have been found to be most contaminated by PCB: the greater scaup (Aythya marila nearctica) and the white-winged scoter (Melanitta deglandi). The 17 samples of scaup had a mean level of 5 ± 7 µg/g (mean ± SD) in the liver, with values ranging from zero to 32 µg/g. Eight samples of scoter had a mean breast-muscle level of 7 ± 67 µg/g, with values ranging from 1.5 to 50 µg/g.

Figure 1 shows the residue patterns of three samples of duck liver. All show contamination by PCB mixtures other than Aroclor 1254, as evidenced by the high relative concentration of some of the later peaks and, in the second scoter sample, of several early peaks also. However, there is a distinct resemblance between these patterns and those of hens contaminated with Aroclor 1254. The ratios of peaks 12:9 in the duck samples are 6, 2 and 10 respectively, indicating that the first two probably result from relatively fresh exposure. Thus the mean notional concentration of 14 µg/g in the first scoter sample may be more embryotoxic than the mean of 50 µg/g in the second sample.

3.3 The rat experiment

The reproductive success of the rats was not affected by the level of PCB intake in this experiment. Intake was terminated after 9 weeks, and the breeding population was observed for a further 16 weeks. Analysis of the tissues of adults, fetuses and pups (both sucklings and weanlings) showed a marked difference in elimination efficiency when compared to the hen. (A detailed report on this study is in preparation.)

Figure 3 shows typical PCB patterns after 3 weeks of exposure and 4 weeks after termination of exposure, i.e., in week 13. Peak 12 does not persist, and only material eluting after that peak could be implicated in any biological effect on the rat. Two liver microsomal enzymes, aniline hydroxylase and cytochrome P450, were significantly activated by the exposure.



Fi ure 3. PCB residue patterns in rats after 3 weeks of intake of Aroclor 1254 and 4 weeks after termination of intake.

The major components of Aroclor 1254 are the components of peaks 9, 12, 13 and 14 (Bush et al. [4]) and of these, only peaks 13 and 14 remain in the rat in the 13th week. These represent 3,4,2',3',4'-pentachlorobiphenyl plus 2,4,5,2',4',5'-hexachlorobiphenyl (peak 13) and 2,3,4,2',4', 5'-hexachlorobiphenyl (Sissons and Welti [6,7]). It is probable that the first constituent of peak 13 is eliminated because it contains the same substitution pattern on one ring as peak 12; hence the two main components of the residue would have the substitution pattern 2,4,5, and both have 4,4'-substitution. The minimal effect of the PCB intake on the rat and its young may be due to the rat's ability to eliminate all but these two compounds, which may themselves be relatively innocuous.

Analysis of the fetus, however, showed that the lack of effect on fetal viability is probably best explained by protection of the developing fetus from PCB contamination by the placenta. The mean PCB concentration in fetuses was 2 μg/g, in sucklings 50 μg/g and in weanlings 20 μg/g during the experiment. Comparison of these levels with the concentrations in the mothers at the same time (e.g. Figure 4) indicates that little PCB reached the fetus. Only the sucklings became contaminated, and by that stage any possible phase of development which was sensitive to attack by PCB must have passed.

4. Conclusions

The work reported here indicates that there are subtle differences in metabolic efficiency for pentachlorobiphenyls with differing substitution patterns between a mammalian and an avian system. It suggests also the importance of the placenta in protecting the fetus from lipophilic agents such as PCBs. It offers the possibility of evaluating the importance of PCB residues of unknown origin found in wildlife specimens by comparison with model laboratory systems.

The levels reported by other workers in a variety of avian species, if they reflect mixture patterns similar to those reported here, may pose a threat to the early stages of development of the species and hence to their reproductive success.

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DISCUSSION

DANIELSON (Sweden)

1. How precisely do you draw conclusions about relative toxicity of the various chlorinated biphenyls from your experiments?

2. Does not the technical PUB used in the experiments contain contaminants?

BUSH (U.S.A.)

1. Embryo-toxicity of the residual PCBs mixture deposited in the eggs by the hens 9 weeks after the termination of exposure was more potent than the residue deposited during the build-up phase of the experiment. Thus 50% embryonic mortality was caused by approximately 50 mg/g PCB at the beginning and by only 10 mg/g during the clearance period. Toxicity in the hen at the latter period must be attributed to the components of peaks 12, 13 and 14, these being the only PCB present at this time. The rat eliminated peak 12, and hence any toxic effects observed must be attributed to peak 13 and 14 material.

2. The technical PCB used in these studies was free of materials such as the dioxins to the best of the author's knowledge.

INFLUENCE DES PESTICIDES ORGANOPHOSPHORES SUR LE METABOLISME DES GRAISSES NEUTRES CHEZ LE RAT

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RESUME

Le problème toxicologique posé par l'utilisation sans cesse croissante des pesticides organophosphorés est l'effet à long terme d'une exposition continue à de faibles concentrations, par exemple sous la forme de résidus alimentaires. Outre l'inhibition des cholinestérases, l'inactivation de nombreuses hydrolases peut être imputée aux esters organophosphorés: c'est notamment le cas d'enzymes importantes impliquées dans le métabolisme des lipides.

Des études effectuées in vitro ont permis la caractérisation, au moyen d'esters organophosphorés, de diverses activités glycéridasiques dans trois tissus du rat (coeur, intestin, et tissu adipeux). Dans le but de vérifier si l'inhibition de ces activités enzymatiques observée in vitro se reproduisait in vivo et, en même temps, d'en examiner les conséquences éventuelles, des animaux ont été soumis (les uns, 3 mois; d'autres, un an) à un ester organophosphoré mélangé à l'alimentation (normale ou enrichie de graisse). Après sacrifice, diverses activités enzymatiques (lipases et cholinestérases) ont été déterminées: divers dosages de constituants des graisses neutres (acides gras, glycérol, cholestérol) ont en outre été effectués.

Ces travaux préliminaires permettent de mettre clairement en évidence l'interférence d'un ester organophosphoré avec le

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métabolisme des graisses neutres in vivo. A des doses où aucun signe clinique d'intoxication ne peut encore être observé, certaines activités lipasiques tissulaires, subissent une réduction. Cette diminution n'a toutefois pas la même importance que celle subie par les cholinestérases, enzymes dont l'activité reste le meilleur témoin d'une exposition excessive aux esters organophosphorés.

ABSTRACT

The toxicological problem raised by the ever-increasing use of organo-phosphorus pesticides is the long-term effect of continued exposure to weak concentrations, for example in the form of residues in food. Apart from inhibition of the cholinesterases, the inactivation of numerous hydrolases can be attributed to organo-phosphorus esters. This is especially the case with certain important enzymes involved in lipidic metabolism.

Various glyceridase activities were observed in vitro in three rat tissues (heart, intestine and adipose tissue), using organo-phosphorus esters. In order to see whether the inhibition of these enzyme activites which had been observed in vitro would be reproduced in vivo and, at the same time, to examine the possible consequences, an organo-phosphorus ester was administered to certain animals(in some cases for three months, others for one year), mixed in with their food (normal or fat-enriched). After they had been killed, various enzyme activities (lipases and cholinesterases) were determined; various quantitative analyses of neutral fat constituents (fatty acids, glycerol, cholesterol) were also carried out.

This preliminary work showed clearly that an organo-phosphorus ester does in fact interfere with the metabolism of neutral fats in the living animal. There is a reduction in certain activities of lipase tissues even with doses which do not give rise to any clinical signs of poisoning. This reduction in activity, however, is by no means as important as that suffered by the cholinesterases, and it is the activity of these enzymes which is the best indicator of excessive exposure to organo-phosphorus esters.

1. Introduction.

L'action des esters organophosphorés sur les estérases agissant préférentiellement sur les esters aliphatiques simples ou les triglycérides à courtes chaînes d'acides gras a fait l'objet de nombreuses études. Au contraire, leur action sur les estérases hydrolysant les glycérides à longues chaînes d'acides gras (en d'autres termes les lipases) est encore peu connue : seule la lipase pancréatique a reçu quelque attention.

Dans un premier temps, nos travaux ont eu pour but la caractérisation des activités enzymatiques responsables de l'hydrolyse des graisses neutres dans les trois tissus du rat (coeur, intestin et tissu adipeux). L'utilisation des esters organophosphorés nous a permis de mettre en évidence dans ces trois tissus des activités mono et diglycéridases. Les résultats de ces travaux ont été publiés [1-4].

Nous nous sommes orientés dans la suite vers l'étude des activités triglycéridases de ces tissus. Nous avons constaté que les deux activités responsables de l'hydrolyse des triglycérides, à savoir la lipase hormonosensible (HSL) et la lipoprotéine lipase (LPL), pouvaient toutes deux être inhibées aussi bien _in vitro_ que _in vivo_ par les esters organophosphorés (résultats non publiés). Il était donc intéressant de vérifier l'effet sur le métabolisme lipidique d'une exposition continue à de faibles concentrations de pesticides organophosphorés.

2. Partie expérimentale.

Cinquante jours après la naissance, des rats femelles (souche Sprague-Dawley) ont été soumis par groupes de dix pendant un an à quatre types d'alimentation dont la base est une farine classique : la farine D03 de la firme U.A.R. (Villemoisson sur Orge, France).

Le régime A est constitué de farine enrichie en matières grasses par addition de 20 % d'huile de maïs ; le régime B contient en plus 10 ppm de l'ester organophosphoré Triamiphos (T = 0) ou 5-amino-(bis diméthylamido) phosphoryl 3-phényl-1,2,4-triazole ; le régime C est de la farine sans aucune addition et enfin le régime D, la farine additionnée de 10 ppm de T = 0. Chaque semaine les animaux ont été pesés et chaque mois du sang a été prélevé pour la détermination de l'activité cholinestérasique érythrocytaire de façon à contrôler l'absorption de l'ester organophosphoré. Les figures 1 et 2 montrent les courbes de poids corporel et l'évolution de l'activité cholinestérasique des globules rouges durant le traitement. L'administration de 10 ppm de Triamiphos permet de maintenir un taux d'inhi-

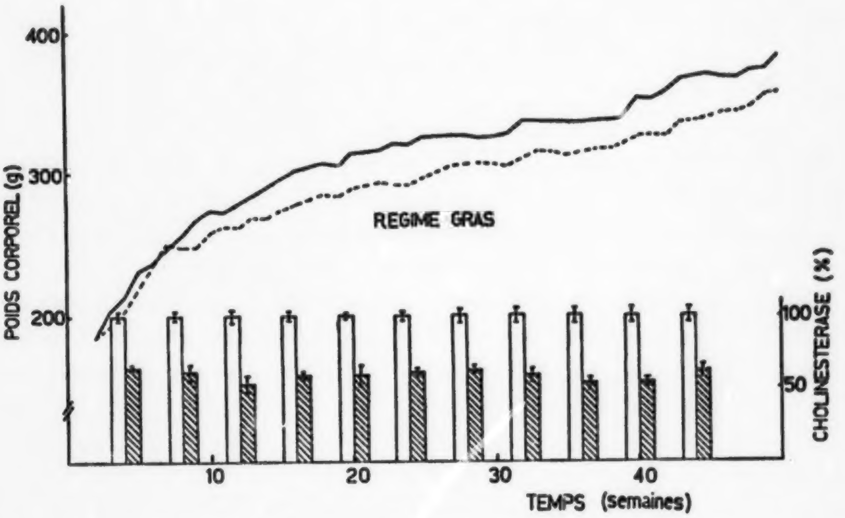

Fig. 1 Courbe de poids et activité cholinestérase érythrocytaire des rats témoins et traités au Triamiphos (régime normal). Exposés (——; ▨) ; controles (- - - - -; ☐).

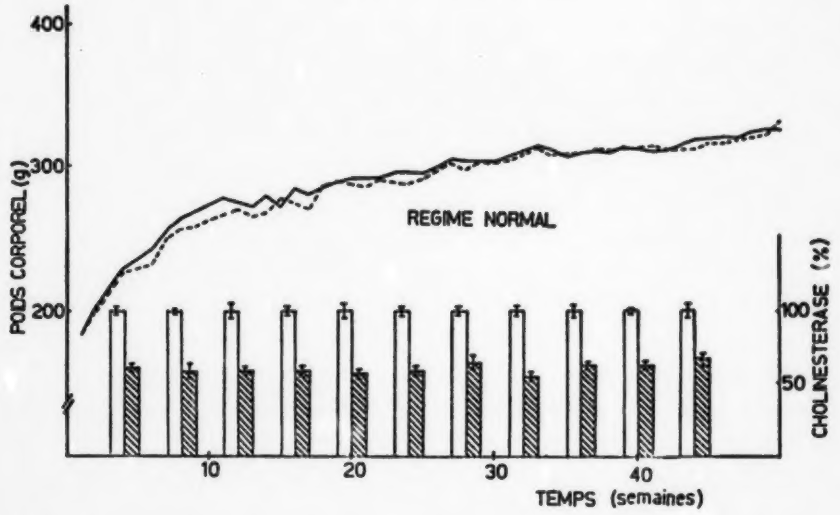

Fig. 2 Courbe de poids et activité cholinestérase érythrocytaire des rats témoins et traités au Triamiphos (régime gras). Exposés (——; ▨) ; controles (- - - - -; ☐).

bition de la cholinestérase du sang total compris entre 40 et 50 % (par rapport à l'activité cholinestérase des animaux témoins correspondants, considérée comme 100 %).

Aucune différence dans l'augmentation de poids n'est observée entre le groupe traité et celui non traité au pesticide, lorsque le régime est à base de farine normale. Par contre, lorsque de l'huile de maïs est ajoutée les animaux soumis au triamiphos ont un poids nettement supérieur à celui des animaux témoins dès la 10ème semaine. Cette différence se maintient pendant toute la période d'observation et ce malgré l'augmentation de poids constatée à partir de la 30ème semaine chez les animaux témoins, soumis au régime riche en graisse (A) par rapport aux témoins soumis au régime normal (C).

A la fin de la période d'exposition, les animaux ont été sacrifiés et las mesures suivantes effectuées :

- activités cholinestérases dans les homogénats de coeur, intestin et cerveau et dans le sérum et le sang total,
- activités mono-, di- et **triglycéridases (HSL et LPL) dans les homogénats** de coeur, intestin et tissu adipeux,
- contenu en acides gras, glycérol et cholestérol totaux des homogénats de coeur et d'aorte et concentration en acides gras libres du sérum.

L'examen de ces résultats, permet de dégager les tendances suivantes:

- après un an de régime riche en graisses ou non, certaines activités lipases ont subi une réduction de l'ordre de 10 à 20 % sous l'influence de 10 ppm de triamiphos (la différence n'est toutefois statistiquement significative avec les deux régimes, qu'en ce qui concerne la monoglycéridase du coeur),
- les activités cholinestérases subissent de plus importantes réductions et chaque fois significatives au seuil de 5 % : ± 40 % pour le coeur et le sang total, ± 25 % pour l'intestin, ± 75 % pour le sérum et ± 10 % seulement pour le cerveau. Cette dernière inhibition très légère, s'explique par le manque de perméabilité de la barrière hémato-encéphalique au métabolite actif du triamiphos [5].
- en ce qui concerne les dosages d'acides gras, glycérol et cholestérol, les constatations suivantes peuvent être faites (tableau 1) :

a. dans le sérum des animaux soumis au régime normal contenant 10 ppm de triamiphos, la concentration en acides gras libres est réduite significativement de 30 %. Cet effet est peut être la résultante d'une

TABLEAU I : Dosage des constituants des graisses après un an de traitement au triamiphos (10 ppm) chez le rat.

Substance dosée	Régime normal (n = 10)	Régime gras (n = 7)
Acides gras libres du sérum	69*+	110
Acides gras totaux dans l'aorte	65	100
Glycérol total dans l'aorte	64	91
Cholestérol total dans l'aorte	122 *	175 *

* $P < 0.05$

+ Résultats exprimés en % du groupe témoin.

réduction de la lipolyse (par inhibition de la HSL) chez les animaux traités par le pesticide. La concentration en acides gras libres du sérum ne change cependant pas chez les animaux soumis au régime gras et recevant du triamiphos.

b. dans les homogénats d'aorte, les concentrations en acides gras et glycérol ne changent pratiquement pas chez les animaux soumis à l'ester organophosphoré et au régime gras alors qu'elle tend à diminuer sans toutefois atteindre le seuil de signification chez les animaux soumis au régime normal et au pesticide : en ce qui concerne les dosages de cholestérol dans l'aorte, on observe, suite à l'administration du triamiphos, une augmentation de concentration de 22 % ($P < 0,01$) chez les animaux soumis au régime normal et de 75 % ($P < 0,001$) chez ceux soumis au régime gras.

3. Conclusion.

Ces travaux préliminaires ont mis en évidence l'interférence d'un pesticide organophosphoré avec le métabolisme des lipides alors qu'aucun signe clinique d'intoxication ne peut encore être observé. Concrètement, cette interférence du triamiphos se traduit dans notre étude par :

- un gain de poids plus important des animaux soumis au régime gras,
- la réduction des activités glycéridases de différents tissus du rat (réduction significative dans le cas de la monoglycéridase du coeur).
- des différences de teneur en constituants des graisses dans le sérum et les homogénats de coeur et d'aorte, et spécialement dans ce dernier tissu, une augmentation nette de la concentration en cholestérol.

Ces observations suggèrent que l'interférence possible des pesticides organophosphorés avec le métabolisme lipidique mérite de retenir l'attention. De telles investigations devraient d'abord être répétées avec d'autres pesticides organophosphorés afin de vérifier si les réponses biologiques observées suite à l'administration prolongée de triamiphos sont communes à d'autres représentants de ce groupe de pesticides.

Ce travail a été financé par le Fonds de la Recherche Scientifique Médicale (projet 1198) et le Fonds de Développement Scientifique (UCL). J.P. Buchet a bénéficié d'un mandat de Chargé de Recherches du Fonds National de la Recherche Scientifique.

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DISTRIBUTION AND METABOLISM OF POLYCHLOROBIPHENYLS

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ABSTRACT

The relation between the structure of the polychlorobiphenyls (PCBs) and their susceptibility to metabolic degradation has been studied in the mouse. The excretion and retention in fat have been investigated with six pure compounds: 3,5,3',5'-tetrachlorobiphenyl (I), 2,5,3',4'-tetrachlorobiphenyl (II), 2,5,2',4',5'-pentachlorobiphenyl (III), 2,3,4,2',4',6'-hexachlorobiphenyl (IV), 2,4,5,2',4',5'-hexachlorobiphenyl (V) and 2,3,4,2',4',5'-hexachlorobiphenyl (VI). Compounds III, IV and V were obtained labelled with ^{14}C*, the excretion of these three was measured over 21 days, and the retention of all six compounds in fat was measured 7 and 21 days after dosing.*

The results obtained with III, IV and V conform with the current view that two vicinal carbon atoms are involved in aromatic hydroxylation; III and IV have such an unsubstituted pair and are not retained in fat and are rapidly excreted, while V, which does not have the unsubstituted pair of carbon atoms, is retained in fat and excreted only very slowly. Compound VI, however, disappears very slowly from fat although it contains a pair of unsubstituted carbon atoms in the 2,3 position, and it is suggested that hydroxylation at this position is blocked by o-chlorine substitution on the other ring, but only if the two rings are approximately co-planar. With three o-chlorine substituents in the molecule, as in IV, the two rings cannot be co-planar, and hydroxylation at the 2,3 position is possible.

The 3,4 position, as in II and III, is available for hydroxylation even with o-chlorines on the other ring. The tetrachloro compound I does not contain two adjacent unsubstituted carbon atoms, and its retention in fat is intermediate between the values obtained with PCBs that are slowly and rapidly excreted. It seems likely that the 3,5-dichlorophenyl ring can undergo a slower metabolism by a mechanism somewhat different than occurs with II, III and IV.

Over 70 different components containing up to 10 chlorine atoms have been detected in the commercial mixtures of polychlorobiphenyls (PCBs). Evidence in the literature is conflicting on the relative rates of biodegradation of different PCBs, but it seems likely that the lower chlorinated components, which form a considerable part of the PCBs liberated into the environment, are preferentially degraded in the lower stages of food webs. Jensen and Sundström [1] have not identified PCBs with fewer than five chlorine atoms in human fat.

Together with other laboratories in Sweden, we are engaged in a programme to assess the risks to health from those PCBs that are absorbed and retained by man; this programme is supported by the National Swedish Environment Protection Board. We have already presented our preliminary results on the distribution and retention in the mouse of 2,5,2',4',5'-pentachlorobiphenyl (Berlin et al. [2]); a PCB which occurs in traces in human fat. This compound is rapidly taken up by the tissues after oral or intravenous administration, mainly by the liver and brown fat, and then it migrates to the general body fat where it reaches a maximum about 24 hr after dosing, at a time when the concentration in other tissues is falling rapidly. A localized higher concentration was maintained in the nasal sinuses and bronchi, and 32 days after the dose the lungs were the most clearly defined organ in a whole-body autoradiogram, with radioactivity located in the bronchial epithelium. Radioactivity is excreted in faeces with a half-time of about 6 days, and we have subsequently shown that the compound excreted is mainly a free and conjugated hydroxy derivative of the PCB.

At a meeting in Stockholm where these preliminary results were discussed, we suggested that the biodegradation of PCBs in the environment might not be influenced primarily by the degree of chlorination, but that it was dependent on the presence of two vicinal unsubstituted carbon atoms in the molecule, a condition more likely to be satisfied in low than in high chlorinated PCBs. This was based on an earlier suggested mechanism for the hydroxylation of chlorobenzenes (Jondorf et al. [3]), and is in line with modern views on aromatic hydroxylation (Jerina [4]). We have now tested this hypothesis by studying the retention and excretion by the mouse of six pure PCBs (Fig 1), supplied by Docent C.A. Wachtmeister of the Wallenberg

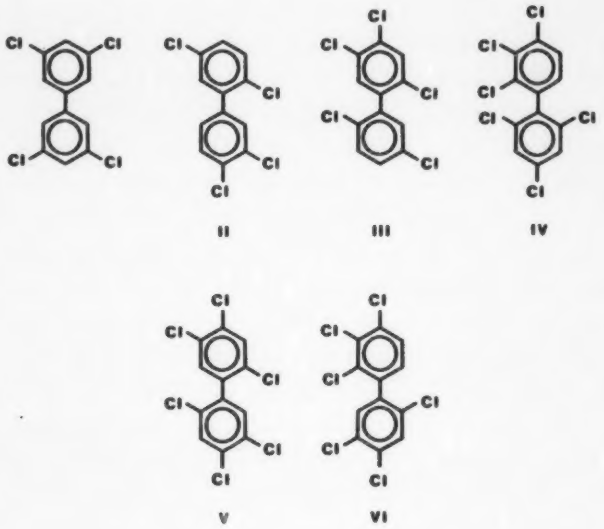


Fig. 1.

Chemical structure of PCBs used in the experiments.

Laboratory, University of Stockholm. Compounds III, IV and V were obtained labelled with ^{14}C.

The six compounds were administered orally in the lipid phase of an aqueous emulsion to groups of 3-6 mice. With the three radioactive compounds the excretion of radioactivity over a 21-day period, and the tissue retention 1, 7 and 21 days after dosing were determined as described in our earlier publication. In addition, all of the compounds were determined in fat 7 and 21 days after dosing by the analytical method of Jensen et al. [5] .

Fig 2 shows the faecal excretion of the three radioactive PCBs III, IV and V, expressed as a percentage of the dose; less than 1% of the dose is excreted in urine. Compounds III and IV, both of which contain a pair of unsubstituted carbon atoms, are excreted with half-times of about 6 days and 1 day respectively, while compound V, which does not contain the pair of carbon atoms, is very slowly excreted. As expected, the differences between the rates of excretion are inversely related to the retention of radioactivity in fat and skin, the main body reservoirs of PCB (Fig 3). The very rapid excretion of IV is not due

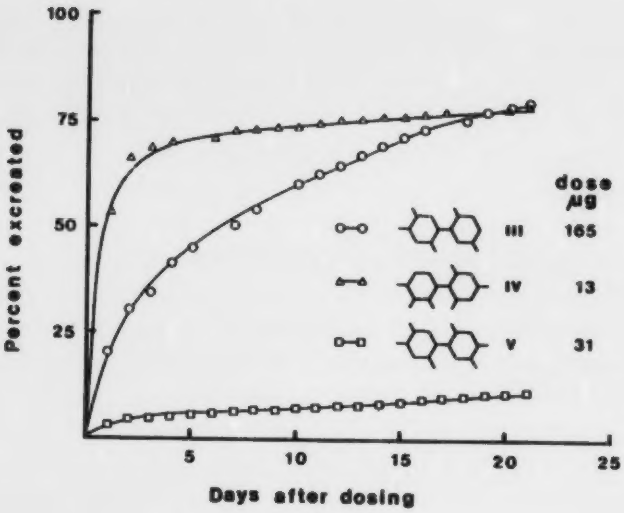
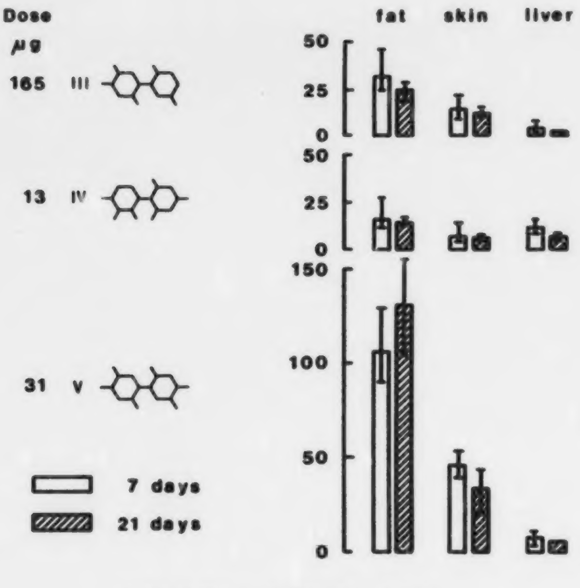


Fig. 2. Excretion of radioactivity in faeces



vertical lines indicate range

Fig. 3. Retention of radioactivity in tissues calculated as PCB equivalent

to incomplete absorption from the gut, as 1 day after dosing the concentration in liver per μg dose was found to be more than double that of the other two, and Fig 3 shows that it remained higher after 7 days. The concentrations of PCB in fat at 7 and 21 days after dosing are shown in Fig 4. The good agreement between the chemical and radioactivity measurements with compounds III, IV and V indicates that all of the radioactivity in fat was present as unchanged PCB. The results with these three compounds support our original hypothesis, but the prolonged retention of hexachlorobiphenyl VI, which contains two unsubstituted vicinal carbon atoms, indicates that this is not the only structural requirement for the metabolism of the PCBs.

The recent investigation of Jensen and Sundström [1] into the retention of PCBs in human fat suggests an explanation of the failure of compound VI to be metabolized and excreted. These authors found that few PCBs with three or four o-chlorine substituents were present in fat, but PCBs with one or two o-chlorines were abundant, and they suggested that o-chlorine substitution may influence metabolic degradation. The structural difference between the two hexachlorobiphenyls IV and VI is IV, which is rapidly metabolized,

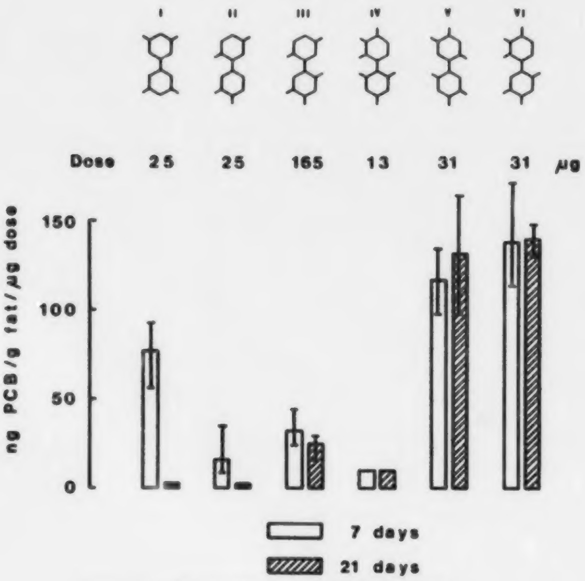


Fig. 4. Retention of PCB in fat

has three o-chlorines, while VI, which is not metabolized, has only two. With two o-chlorines the biphenyl molecule can assume a co-planar configuration while with three o-chlorines it cannot, and it seems likely that o-chlorine substitution can inhibit hydroxylation in the 2,3 position of the other ring, but only if the two rings are co-planar.

Jensen and Sundström found traces of III in human fat, and rather more of 3,4,2′,3′,6′-pentachlorobiphenyl, and they concluded that two o-chlorines could depress hydroxylation in the 3,4 position. Our investigation has indicated that III is fairly rapidly metabolized in the mouse, so the o-chlorine substituents have little or no influence on this pair of carbon atoms. The concentration in fat is, however, a function of not only the rate of absorption but also of the rate of intake, and it is possible that these two pentachlorobiphenyls constitute a considerable proportion of the PCBs in human diet, although positive evidence of this is not available.

The low retention of the tetrachlorobiphenyl II in fat is to be expected from the presence of a 3,4 pair of unsubstituted carbon atoms, but I, which contains no such pair, shows a retention in fat that is intermediate between that of II and of the slowly metabolized hexachlorobiphenyls V and VI. Jondorf et al. [3] have shown that 1,3,5-trichlorobenzene gives a small proportion of a hydroxy derivative, and it is possible that the 3,5-dichlorophenyl group does not undergo the rapid metabolism associated with a vicinal unsubstituted pair of carbon atoms, but that degradation proceeds by an alternative slower mechanism.

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DISCUSSION

DANIEL (U.K.)

1. Have you determined the level of glutathione in the liner following the administration of the various polychlorobiphenyls?

2. Does the degree of ortho-substitution affect the difference spectrum given with microsomal suspensions.

HOLM (Sweden)

The present investigation has been limited to a study of the relation between the structure of the PCBs and their excretion and retention in fat. It is hoped that it may be possible to extend the investigation to include a more detailed study of the mechanism of biodegradation. The point raised by Dr. Daniel will be considered at that time.

RELATION OF THE PHYSICAL/CHEMICAL STATE OF A PLASTICIZER, DI-(2-ETHYLHEXYL)PHTHALATE (DEHP) TO ITS BIOLOGICAL DISPOSITION AND ACTION

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ABSTRACT

DEHP, a highly water-insoluble plasticizer which has been shown to be widely distributed in the environment as well as to be a contaminant of human blood stored in vinyl plastic bags, was prepared in 3 different vehicles. Each preparation represented a different type and degree of oil-in-water dispersion. These were respectively: (1) a sonicated, aqueous emulsion which was milky white and opaque, (2) a detergent-dispersed suspension in 50% DMSO, 5% Tween 80 in saline which was opalescent, and (3) a detergent-solubilized preparation in 25% DMSO, 10% Tween 80 in saline which was completely transparent and, microscopically, exhibited no droplets. DEHP (250mg/kg iv) in each of these 3 preparations, had a markedly different effect on in vivo reticuloendothelial (RE) function in rats, implicating the physical state of the DEHP in the RE effect. There appeared to be an optimal droplet size for maximal inhibition of RE function with (3) having no significant effect. The kinetics of disappearance from blood and distribution were also markedly different for DEHP in (1) and (3). In (1), it disappeared biexponentially and accumulated primarily unchanged in the liver while in (3) it disappeared monoexponentially and accumulated primarily in the eviscerated carcass. DEHP, solubilized in Tween 80, resulted in a respiratory distress syndrome and lethality due to an acute alveolar inflammatory response which was not seen with either compound alone.

(Supported by grants ES34, ES 454 and ES 44887 from NIEHS and contracts NAS-5-22071 from NASA and 72-29903 from NHLI).

1. INTRODUCTION. The annual American production of phthalate ester plasticizers is approximately one billion pounds [1]. With recent evidence of its widespread environmental distribution and contamination of human blood stored in plastic bags, considerable interest has centered on its potential toxicologic hazard [2]. Because DEHP, the most widely used phthalate, is a water-insoluble oil, we have been interested in its disposition and biological effect when administered in various vehicles that result in different physical states. Three different formulations have been studied: (1) a sonicated emulsion in 3% gum acacia (DEHP=50 mg/ml). This preparation is milky white and completely opaque. Under phase contrast microscopy it exhibits 19×10^6 droplets/ml with a diameter of 1.9-2.6μ, (2) a detergent-dispersed suspension in a vehicle of 50% DMSO and 5% Tween 80 in saline (DEHP=100 mg/ml). This preparation is opalescent and contains 5.4×10^6 droplets/ml of diameter 10.5-15μ. (3) a detergent-solubilized preparation in a vehicle of 25% DMSO and 10% Tween 80 in saline (DEHP=50 mg/ml). This preparation is completely transparent and under phase contrast microscopy exhibits no visible droplets. Extensive Brownian movement suggests submicron solubilized micelles of DEHP. Dilution of these 3 preparations with rat plasma to an extent that mimics their *in vivo* dilution results in the settling out of additional oil droplets with the first two preparations but has no effect on the solubility of DEHP in the third.

2. RESULTS. In Table 1 are shown the effects of the three different formulations of DEHP on *in vivo* reticuloendothelial function in rats. The dose-response curve for formulation (1) is shown for doses of DEHP between 125-500 mg/kg. There is no significant alteration at 125 mg/kg with the maximum inhibitory effect seen at 250 mg/kg. At this latter dose the

detergent-dispersed preparation (formulation (2)) produces an approximately 2.5 fold greater effect while the detergent-solubilized preparation (formulation (3)) results in no significant alteration. These results clearly implicate the particle size and number in the RE effect and indicate no direct inhibitory effect of DEHP.

TABLE 1.

EFFECT OF DEHP ON RETICULOENDOTHELIAL FUNCTION IN RATS

DOSE mg/kg iv	% CHANGE IN CLEARANCE HALF-TIME FORMULATION (1)	(2)	(3)
125	- 9 ± 5	-	-
250	+75 ± 5*	+210 ± 10*	+10 ± 5
500	+77 ± 5*	-	-

Twenty-four hours after the administration of the indicated doses of DEHP or appropriate vehicle for each formulation, a test dose of carbon particles was injected i.v. and the clearance of the carbon from the blood was followed. The carbon clearance was monoexponential. Half-time was calculated from the rate of clearance and the data are shown as the mean % change in clearance half-time from the respective vehicle-injected controls ± S.E.M. Increases in clearance half-time represent paralysis of RE function.

* = p<.001

The clearance rates of both emulsified and solubilized DEHP are shown in Table 2. The emulsified DEHP disappears biexponentially with rate constants for the initial rapid α phase and slower β phase of .200 and .021 min^{-1}, respectively. In addition, approximately 90% of the injected dose disappears from the blood during the α phase. The solubilized DEHP disappears monoexponentially with a rate constant of .036 min^{-1}, which is not significantly different from that of the β phase for the emulsified DEHP. No rapid distribution phase is seen.

TABLE 2.

KINETICS OF DEHP DISAPPEARANCE FROM BLOOD

FORMULATION	FIRST-ORDER RATE CONSTANT (min^{-1}) ($\bar{x}$±SEM)	
	α phase	β phase
Emulsion in 4% BSA	.200 ± .082	.021 ± .017
Detergent-solubilized	—	.036 ± .055

^{14}C-labelled DEHP at a dose of 200 mg/kg was injected i.v. in the indicated formulation. At 2, 5, 15, 30, 45, and 60 minutes, blood samples were taken and the amount of unchanged DEHP remaining in the blood was determined. After 1 hour approximately 5% of the initial concentration of the emulsified DEHP and 20% of the solubilized DEHP remained in the blood. In these experiments DEHP was emulsified in 4% bovine serum albumin (BSA). Visually and microscopically this preparation was similar to the one in 3% gum acacia. Preliminary experiments indicate that the in vivo rates of disappearance from blood are also similar.

In Table 3 is shown the distribution of emulsified and solubilized DEHP at 1 and 24 hours after i.v. injection. It can be seen that the liver contains 48.4% of the emulsified dose as unchanged DEHP after 1 hour; after 24 hours this organ still retains 9.6% of the dose. In contrast, at 1 and 24 hours the liver contains only 11.3 and 0.2% of the solubilized dose, respectively. Likewise, significant amounts of the emulsified DEHP are found in the spleen at 1 and 24 hours while only small or trace amounts of the solubilized DEHP are recovered in that organ. In contrast to the preferential uptake of the emulsified DEHP into liver and spleen, the solubilized DEHP is recovered primarily in the eviscerated carcass (i.e. skeletal muscle and bone). Note the lack of a significant difference in the distribution of the two forms of DEHP into lung. In spite of its lack of a major accumulation in the liver, the solubilized DEHP is readily metabolized to water-soluble metabolites. In fact, after 1 hour there is a slightly but significantly greater accumulation of metabolites in the liver, and after 24 hours a significantly greater amount of the solubilized DEHP is recovered in the urine and feces as metabolites.

During these experiments we noted that rats given the detergent-solubilized DEHP die in respiratory distress at doses of DEHP which have no overt effects when administered as aqueous emulsions. The respiratory distress begins almost immediately and death occurs within 90 minutes. At autopsy the lungs are grossly enlarged and covered with dark, hemorrhagic patches. The DMSO, Tween 80-containing vehicle alone does not produce the pulmonary damage and subsequently we found that DMSO could be left out of the preparation without

TABLE 3.
TISSUE DISTRIBUTION OF 2 DOSAGE FORMS OF DEHP
(% of injected dose: 200 mg/kg iv)

	EMULSIFIED		SOLUBILIZED	
	% recovered as DEHP (±SE)			
	1 hr.	24 hrs.	1 hr.	24 hrs.
LIVER	48.4±5.2	9.6±1.4	11.3^{**} ±0.5	0.2^{**} ±0.05
CARCASS	12.1±3.1	2.9±0.2	33.5^{**} ±1.7	12.4^{**} ±4.0
SPLEEN	6.1±1.0	1.8±0.4	0.6^{**} ±0.08	TRACE^{**}
LUNG	1.2±0.1	0.3±0.02	1.3 ±0.2	0.2 ±0.05
	% recovered as H_2O-soluble metabolites (±SE)			
	1 hr.	24 hrs.	1 hr.	24 hrs.
LIVER	1.7±0.3	1.2±0.3	3.0^{*} ±0.3	0.5 ±0.05
CARCASS	1.6±0.6	0.2±0.1	10.9^{**} ±1.8	0.6 ±0.2
EXCRETA:	4.3±1.6	53.2±2.8	6.2 ±0.5	66.9^{*} ±4.3
Intest. cont.				
Feces				
Urine				

$^{*}p<0.05$ or $^{**}p<0.01$ when compared to corresponding emulsified distribution altering the respiratory syndrome and lethality. Table 4 shows the effect of Tween 80-solubilized DEHP on lung weight/body weight ratios and lethality in rats. The varying doses of DEHP were achieved by injecting various volumes of the same concentration of DEHP in 13.3% Tween 80. Each dose level was controlled by a group of rats receiving an equal volume of vehicle alone. The vehicle alone produces no significant alterations in lung weight/body weight ratios when compared to non-injected controls. In addition, no deaths are seen with the vehicle-injected controls. However, a clear dose-response relationship can be seen for lung weight/body weight

TABLE 4.

LETHALITY OF TWEEN 80-SOLUBILIZED DEHP AND EFFECT ON LUNG WEIGHT IN RATS

	LETHALITY No. Dead/No. Treated		WET LUNG WT/BODY WT ($\bar{x}$ ± SEM)x10^3	
DEHP Dose mg/kg	vehicle injected controls	DEHP treated	vehicle injected controls	DEHP treated**
200	0/10	0/10	5.02±0.15*	5.85±0.17+
250	0/8	4/17	4.78±0.14*	6.18±0.36+
300	0/13	10/15	4.75±0.12*	7.17±0.62+

*Not significantly different from non-injected controls (4.69±0.16)

**Data reported only for animals surviving for 90 minutes

+Statistically different from corresponding vehicle-injected controls (p<.01) ratios and lethality as a function of DEHP dose. The acute LD_{50} for the DEHP:Tween 80 solution lies between 250-300 mg/kg. At 150 mg/kg there are no overt symptoms and the lung weights are not significantly different from controls. We have further observed that the administration of 300 mg/kg of corn oil or another closely related plasticizer, di(2-ethylhexyl) sebacate solubilized in Tween 80 produces no lung alterations nor deaths. Thus, the results shown in Table 4 must be due to some specific interaction between the micellar DEHP and Tween 80. Histologically, vehicle-injected lungs are indistinguishable from non-injected control lungs. The pulmonary lesion seen with solubilized-DEHP is due to an acute alveolar inflammation and is characterized by a dramatic thickening of the intra-alveolar septa arising from the migration of polymorphonuclear leukocytes from the capillaries. As the acute pathology progresses, other blood elements can be seen in the alveolar walls; occassionally, migration across the wall into the air spaces themselves can also be seen. Time course studies indicate that the pathological lesion is fully developed within 15 minutes after injection and, in animals that survive, can be seen for at least 50 hours. In addition, the lesion can also be seen histologically following doses of DEHP as low as 50 mg/kg.

3. CONCLUSION. These experiments demonstrate that alteration of the physical state of the dosage form of DEHP leads to differences in biological activity, kinetics of disappearance from the blood, tissue distribution, and, perhaps most importantly, unmasks a type of pathology not previously seen. These results emphasize the necessity for knowledge of the physical state of DEHP in evaluating its toxicologic potential and signals a concern for the interaction with Tween surfactants that are so readily available in the human diet.

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DISCUSSION

De BRUIN (Netherlands)

Two questions:

. Type of metabolic change of DHEP and structure of metabolites?

. May DEHP be categorized as a microsomal enzyme inhibitor in iew of its effects upon barbital sedative action?

UBIN (U.S.A.)

. First a mono-deesterification of the DEHP to form the monoester, EHP. This is followed by oxidation of the terminal carbons of the emaining side-chain to form the alcohol, ketone and terminal carboxyl roup. This is followed by B-oxidation that results in a shortening he sidechain by 2 carbons.

. Although DEHP prolongs hexabarbital sleeping time, our studies ndicate that it does not do so by inhibiting the microsomal meta- olism. It apparently affects the distribution of the hexabarbital o the brain or perhaps even brain sensitivity to the bariturate.

USH (U.S.A.)

Did you measure passage of your emulsions and mycellular ispersions through the GI tract?

RUBIN (U.S.A.)

Following the oral administration of DEHP in corn oil, we have observed the almost quantitative excretion of the DEHP as water soluble metabolites in the urine and feces. The presence of these metabolites in urine indicates the passage of either the DEHP or its metabolites through the systemic circulation. However, we have not been able to find any evidence of DEHP or its metabolites in the blood following oral administration (single intragastric dose). We interpret these results to mean that the rate of GI absorption is slow while metabolic conversion and urinary excretion of metabolites are rapid. This would tend to minimize the accumulation of either DEHP or its metabolites in the systemic circulation.

THE EFFECT OF MERCAPTODEXTRAN AND N-ACETYLHOMOCYSTEINE ON THE EXCRETION OF MERCURY IN MICE EXPOSED TO METHYL MERCURIC CHLORIDE

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ABSTRACT

In order to prevent the reabsorption of biliary excreted mercury in methyl mercuric chloride exposed mice, the animals were treated with a macromolecular polythiol (Mercaptodextran) synthesized by thiolating a dextran compound using N-acetylhomocyteine thiolactone.

The polythiol given in food at a concentration of 5% reduced the average biological half time of mercury from 11.6 days to 5.9 days. Faecal excretion of mercury compounds was not changed during 10 days, but urinary excretion increased by a factor of 5.

The effect of the macromolecule seems to be related to N-acetylhomocysteine being released in the gastrointestinal tract. N-acetylhomocysteine thiolactone had the same effect as the macromolecule. Corresponding results could be obtained by intravenous injection of N-acetylhomocysteine. The mechanism seems to include the formation in the gastrointestinal tract and in the blood of a N-acetylhomocysteine-methyl-mercuric complex. This complex is easily absorbed when formed in the gastrointestinal tract, and it is rapidly excreted in the urine.

1. Introduction.

Biliary excretion of methyl mercuric salts is higher than faecal excretion indicating intestinal reabsorption (Norseth, Clarkson [1]). A sulphydryl substituated polyvinyl has been demonstrated to increase the elimination probably by complexing mercury in the gastrointestinal tract (Clarkson, Small, Norseth [2]). The aim of the present investigation is to study the effect of mercaptodextran, a thiolated dextran of high molecular weight (Jellum, Aaseth, Eldjarn [3]), on mercury excretion and retention after exposure of mice to methyl mercuric chloride.

2. The effect of Mercaptodextran on excretion of mercury.

Female mice weighing about 20 g were given a single intravenous injection of 5 μmol Hg/kg as radiolabelled methyl mercuric chloride. Mercaptodextran (SH-500) was synthesized from dextran with average molecular weight of 500.000 (Eldjarn, Jellum [4]). Ten mice were given a diet containing 5% SH-500, and 10 mice served as controls. The animals were started on the diet to be tested 48 hours prior to the injection of methyl mercuric chloride. Radioactivity was determined every other day in the whole animal using a whole body counter. The semilogarithmic graphs of the body burden of mercury in control and treated animals is seen to be approximately linear over the time period of observation. Accordingly a constant fraction of the body burden was excreted per time unit, and the biological half-times ($t_{\frac{1}{2}}$) of mercury can be calculated according to the formula

$B_t = B_o \cdot e^{-\lambda t}$ where $\lambda = \ln 2/t_{\frac{1}{2}}$

B_o = initial body burden of mercury

B_t = body burden of mercury after a time period t

Mercaptodextran reduced the biological half-time of mercury after exposure to methyl mercuric chloride from 11 days in control animals to 5.9 days in the treated animals (Fig. 1).

Thus Mercaptodextran decreased the biological half-time of mercury by a factor of two, which equalized the reduction previously shown for a polyvinyl resin (Clarkson, Small, Norseth [2]).

During the treatment with Mercaptodextran radioactivity in

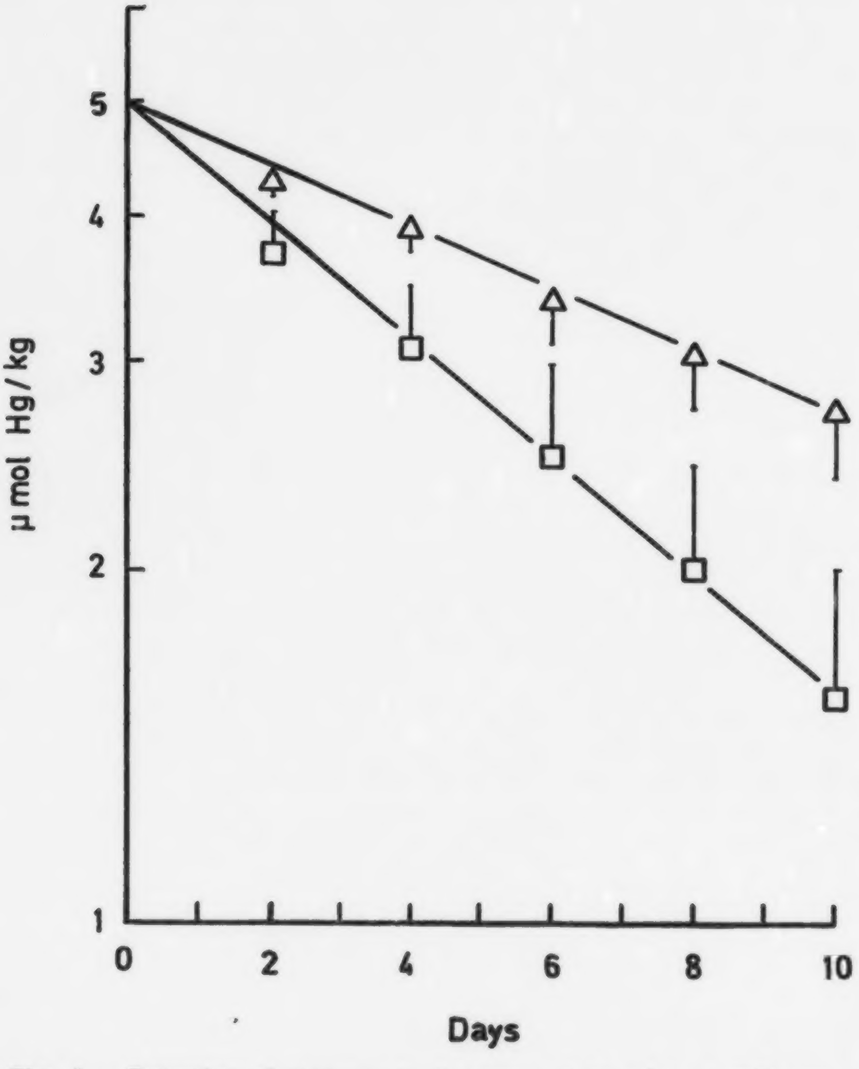

Fig. 1 Retention of mercury in 2 groups of 10 mice at different time intervals after a single intravenous injection of methyl mercuric chloride. Triangles indicate mean values in the control group, and squares indicate values in the group treated with food containing 5% mercapto-dextran (SH-500). Standard deviation is given in the figure.

faeces and urine was determined daily. The total amount of mercury excreted in faeces and urine during 10 days is shown in Fig. 2. Faecal excretion of mercury unexpectedly did not change as a result of treatment with SH-500. Mercaptodextran, however, increased the urinary output of mercury by a factor of 5. About 40% of the dose given was recovered in the urine of experimental animals versus about 8% in the urine of controls.

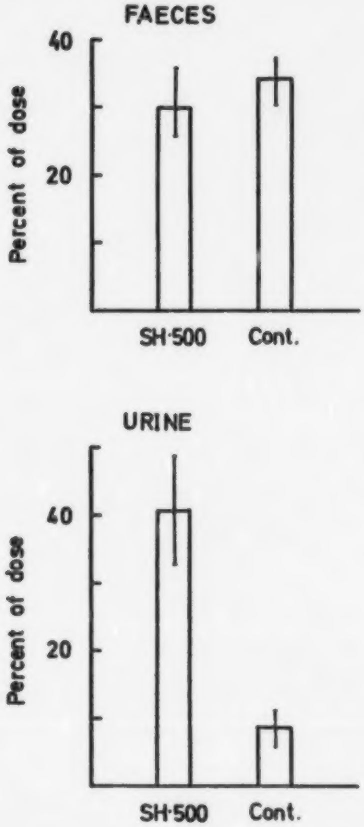

Fig. ·2 Mercury excretion for 10 days in faeces and urine from the same groups of mice after a single intravenous injection of methyl mercuric chloride.
The bars denoted SH-500 and cont. represent mean excretion from mice treated orally with SH-500 and from the controls, respectively. Experimental range is indicated.

3. The effect of N-acetylhomocysteine and its thiolactone on excretion of mercury.

As the synthesis of Mercaptodextran involves the use of N-acetylhomocysteine thiolactone, the latter compound was mixed in the diet of a mouse group in a concentration of 10 µmol/g food. The thiolactone appeared to have an effect almost similar to that of Mercaptodextran.

The similar effect of Mercaptodextran and N-acetylhomocysteine thiolactone indicates that the active agent in both cases is N-acetylhomocysteine. The thiolactone is easily hydrolyzed, and the macromolecule SH-500 (= N-acetylhomocysteinylaminoethyl-dextran) may well release N-acetylhomocysteine in the gastrointestinal tract by enzymatic cleavage.

In order to identify the active agent, groups of mice were given intravenous injection of 500 µmol/kg of SH-20 (mercaptodextran, average molecular weight 20.000), N-acetylhomocysteine thiolactone or N-acetylhomocysteine immediately after the mercury injection, 6 mice served as controls. Urinary excretion of mercury was increased by a factor of about 5 when N-acetylhomocysteine was given intravenously (Fig. 3). Treatment with SH-20 or N-acetylhomocysteine thiolactone was less efficient.

The latter experiment supports the hypothesis that N-acetylhomocysteine released in the gastrointestinal tract is responsible for the effect of Mercaptodextran. Thus, in the gut a complex may be formed which is different from that normally excreted in the bile. This compound, probably a methyl mercuric-N-acetylhomocysteine complex is, in the same way as the biliary complex, easily reabsorbed, but is rapidly excreted in the urine.

Provided that only the biliary excreted mercury in the gut is available for complexation, the highest estimated effect is a reduction of the biological half-time of mercury by a factor of 4. By increasing the dose of N-acetylhomocysteine thiolactone to 10 and 30 µmol/kg per day given in the food the biological half-time was reduced to 1.3 and 1.1 days respectively, versus 8.1 days in the controls. This reduction in the biological half-time by a factor of about 7 is incompatible with the hypothesis of complexation only of biliary excreted mercury in the gut. The formation

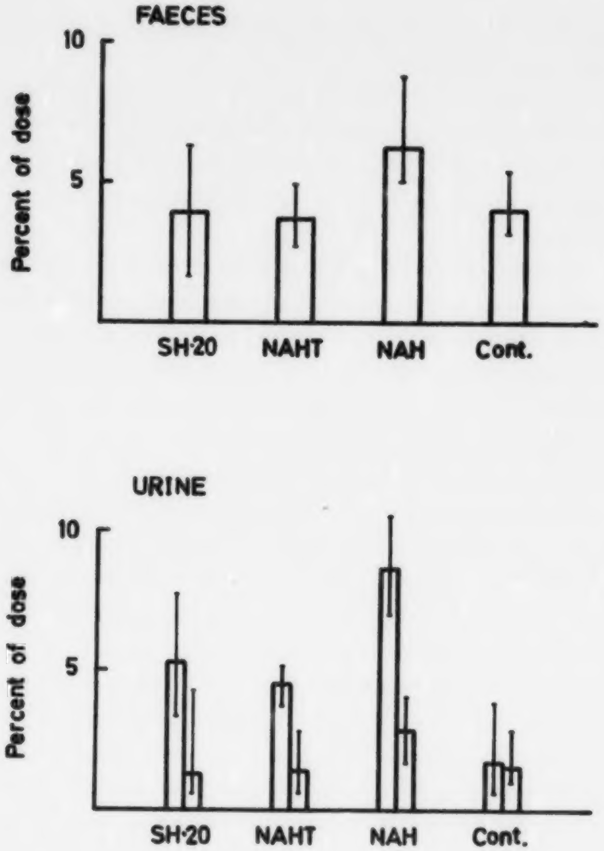

Fig. 3 Mercury excretion for 2 days in faeces and urine from mice after a single intravenous injection of methyl mercuric chloride. The bars denoted SH-20, NAHT, and NAH represent mean excretions from 12, 6, 8, and 6 mice, treated intravenously with 500 µmol/kg of mercaptodextran (SH-20), N-acetylhomocysteine thiolactone or N-acetylhomocysteine, and the column denoted cont. represents excretion from 6 control animals. The bars showing urinary excretion indicate values for the first and second day. Experimental range is indicated for each group.

of a rapidly excreted complex, possibly between methyl mercury and N-acetylhomocysteine, accordingly is thought to occur in the blood as well as in the gut.

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POLLUTION DES ALIMENTS PAR LES HYDROCARBURES PARAFFINIQUES: ETUDE DU DEVENIR DE CES SUBSTANCES CHEZ LES ANIMAUX SUPERIEURS

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RESUME

L'étude du métabolisme des hydrocarbures aliphatiques chez les mammifères présente un intérêt certain, à la lecture de plusieurs travaux récents, faisant état de l'augmentation très nette depuis 1940 des cas de lipidose folliculaire de la rate et de l'accumulation d'hydrocarbures dans les nodules lymphatiques chez l'homme. Des hydrocarbures aliphatiques ont également été isolés du tissu artériel et des plaques athéromateuses chez l'homme. Des lésions, scléroses et dégénérescences ont été provoquées chez le rat et le lapin après ingestion prolongée de doses importantes d'huiles minérales. Les hydrocarbures isolés des tissus humains ont pu être identifiés comme provenant de l'ingestion d'huiles minérales qui seules renferment des cycloparaffines, en revanche, les n-alcanes peuvent provenir également des aliments, les végétaux renfermant à l'état naturel de 0,001 à 0,1% de n-alcanes lourds (C_{17} à C_{31}).

En dehors de tout usage thérapeutique ou diététique, la quantité annuelle d'hydrocarbures ingérés par individu a été évaluée à 400g aux Etats-Unis. La FDA a évalué à 50g/an/individu la quantité d'huiles minérales ingérées. L'origine de ces dernières se situe au niveau des traitements technologiques alimentaires (enrobage des fruits, revêtement intérieur des emballages alimentaires en carton, agent de démoulage ...).

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Le développement récent de la production de levures d'alcanes destinées à l'alimentation animale et plus tard sans doute à l'alimentation humaine pose un problème analogue. Ces levures, lorsqu'elles sont cultivées sur gas-oil, contiennent des résidus d'hydrocarbures (cyclo et iso-paraffines) dont le taux maximum autorisé est de 0,08%. Des sources nouvelles de protéines, telles les algues spirulines, contiennent des quantités non négligeables d'hydrocarbures (0,15% d'heptadécane).

Les travaux suivants sont présentés:

- Une étude de l'absorption des différentes paraffines et des quantités résiduelles non métabolisées, fixées dans la carcasse de l'animal.

- Une expérience destinée à suivre l'accumulation de l'heptadécane dans les carcasses et les différents tissus de rats recevant un régime où les spirulines constituent la source unique de protéines.

- Une étude plus fine du stockage réalisée, également sur des rats, à la suite de l'ingestion d'un régime alimentaire renfermant une dose élevée de dodecylcyclohéxane. Un niveau de saturation des tissus en ce produit ayant été atteint, l'évolution de la quantité stockée a été étudiée chez des rats soumis à un régime amaigrissant.

ABSTRACT

Several recent studies noting the very distinct rise since 1940 in the incidence in humans of follicular lipidosis of the spleen and hydrocarbon accumulation in the lymph nodules are of considerable interest in the study of the metabolism of aliphatic

hydrocarbons in mammals. Aliphatic hydrocarbons have also been isolated in human arterial tissue and atheromatous plaques. Prolonged ingestion by rats and rabbits of considerable quantities of mineral oils produced lesions, scleroses and degenerative effects. It has been possible to identify the source of hydrocarbons isolated in human tissues as the ingestion of mineral oils, which are the only source of cycloparaffins. On the other hand, n-alkanes can also come from food, since plants contain in their natural state between 0.001% and 0.1% (C_{17} to C_{31}) of heavy n-alkane

The annual hydrocarbon intake per person, apart from that taken for therapeutic or dietetic purposes, has been estimated in the United States as 400g. The FDA has estimated the intake of mineral oils per person per year as 50g, the source of which is to be found in technological food processing (wrappings of fruit, inner lining of cardboard food containers, non-stick coatings, etc.).

A similar problem is created by the recent development of alkane yeast production for animal feeding and later, doubtless, for human food. When these yeasts are cultured on fuel oil, they contain hydrocarbon residues (cyclo- and iso-paraffins), the maximum permissible proportion of which is 0.08%. New sources of proteins, such as spiruline algae, contain not inconsiderable amounts of hydrocarbons (0.15% heptadecane).

The following studies are presented:

- *A comparative study of the absorption of different paraffins and non-metabolized residues in the animal carcass.*
- *An experiment to trace the accumulation of heptadecane in the carcasses and different tissues of rats given a diet in which spirulines constitute the sole source of proteins.*
- *A closer study of the storage in rats given a diet with a large dodecylcyclohexane content. When the saturation of the tissues with this substance reached a certain level, the development of the quantity stored was studied in rats subjected to a reducing diet.*

Introduction

Les hydrocarbures aliphatiques ont été mis en évidence chez les mammifères il y a déjà longtemps, mais le rôle biologique de ces substances, souvent considérées comme inertes, n'est pas clair. De nombreuses études effectuées sur le Rat, le Poulet, les ruminants et les poissons ont permis de déceler des n- et des cyclo-alcanes ainsi que des paraffines ramifiées au niveau de divers organes et tissus, et en particulier dans le foie, la rate et l'encéphale. Les paraffines sont également présentes chez l'Homme où leur existence a été signalée dans la rate, le foie, les ganglions lymphatiques mésentériques, les ganglions du système porte hépatique, les tissus de l'artère fémorale et dans les méninges.

L'absorption des hydrocarbures aliphatiques est maintenant bien admise, aussi attribue-t-on leur présence chez les animaux supérieurs à une origine alimentaire (végétaux en particulier) et, en ce qui concerne les cycloparaffines, aux contaminations des aliments par les huiles minérales. Les quantités ingérées par l'Homme sont en augmentation constante, compte tenu d'une part de l'usage des huiles de paraffine en technologie alimentaire (enrobage des fruits, revêtement intérieur des emballages alimentaires en carton, agent de démoulage), à des fins thérapeutiques ou dans des régimes basse-calorie, et d'autre part, des hydrocarbures accumulés chez diverses espèces animales dont nous consommons les tissus.

Le développement récent de la production de levures d'alcanes destinées à l'alimentation animale et plus tard sans doute à l'alimentation humaine, pose un problème analogue. Ces levures, lorsqu'elles sont cultivées sur gas-oil, contiennent des résidus d'hydrocarbures (cyclo et isoparaffines) dont le taux maximum autorisé est de 0,08 %. Enfin, des sources nouvelles de protéines, telles les algues spirulines, contiennent des quantités non négligeables d'hydrocarbures (0,15 % d'heptadécane).

L'ingestion d'origine alimentaire a été évaluée à 400 g/tête/an aux U.S.A., quantité à laquelle il faut ajouter 50 g d'huiles minérales. On peut se demander quelles sont les répercussions de telles quantités ingérées sur l'organisme humain. En effet, BOITNOTT et MARGOLIS ont établi une corrélation entre la quantité d'inclusions sous forme de gouttelettes dans le foie voire même la dégénérescence des cellules hépatiques et la quantité mesurée d'hydrocarbures dans ce tissu ; d'autre part, ils ont mis en évidence une relation entre le nombre de cas de lipidose folliculaire de la rate et l'utilisation ou la consommation croissante d'huiles minérales depuis 1940.

LIBER et ROSE, étudiant les cas de lipidose folliculaire, ont constaté que les rates de sujets atteints étaient plus riches en isomères ramifiés tandis que les rates normales contenaient essentiellement des n-alcanes. CAIN a montré que les méninges de sujets atteints de méningiomes renferment des quantités de paraffines plus importantes que celles de sujets sains. Une constatation identique a été faite au niveau de l'aorte de lapins souffrant d'athérosclérose. De plus, des lésions, scléroses et dégénérescences ont été provoquées chez le Rat et le Lapin après ingestion prolongée de doses importantes d'huiles minérales. Enfin,l'utilisation accrue de pesticides ou de produits agissant en synergie avec les pesticides, peut être à l'origine de l'augmentation des paraffines tissulaires chez les mammifères, du fait de leur rôle inhibiteur des processus de détoxification microsomale.

Il existe quelques travaux concernant l'absorption d'huiles minérales et des différentes classes d'alcanes chez le Rat et les ruminants. Les taux de rétention apparente (ingéré moins excrété) ont été déterminés pour différentes longueurs de chaîne et les voies d'absorption établies.

Nous rapportons ici les travaux concernant l'absorption, la rétention et l'accumulation chez le Rat de divers hydrocarbures paraffiniques.

I. Bilan métabolique et rétention

Les expériences ont porté sur :

- Cinq n-paraffines	Heptadécane
	Eicosane
	Hénéicosane
	Tétracosane
	Dotriacontane
- Deux cyclo-paraffines	Dodecylcyclohexane
	Heptadecylcyclohexane
- Deux paraffines ramifiées	2,2,4,4,6,8,8, heptaméthylnonane
	2,6,10,14 tétraméthylpentadécane (Pristane)

Pour chaque hydrocarbure étudié, huit rats femelles de 150 g environ reçoivent individuellement au jour 0 une dose de 15 mg du produit considéré, incluse dans leur nourriture en solution dans l'huile d'arachide entrant dans la ration. Urines et fèces de trois rats sont collectés pendant dix jours tandis que pour l'étude de la rétention, les animaux sont abattus respectivement 1,2,3,5,7,10,15 et 21 jours après l'administration.

Les conclusions de ces premières études sont les suivantes :

Les hydrocarbures envisagés n'ont donné lieu à aucune élimination urinaire. En revanche, il existe une élimination fécale qui donne une idée du niveau de rétention apparente. Ces résultats ne permettent pas de dégager de ligne générale applicable à l'ensemble des paraffines et ce d'autant plus qu'il existe une assez grande variabilité individuelle. Toutefois, il ressort que la rétention apparente est très élevée, de l'ordre de 90 à 95 p.100, tant que le nombre d'atomes decarbone reste inférieur à 24.

En ce qui concerne la rétention du produit inchangé au niveau de la carcasse, les résultats obtenus permettent de diviser les paraffines en deux catégories :

- d'une part, les isoparaffines qui semblent avoir un comportement particulier : bien que donnant lieu à une rétention apparente importante, elles ne sont à l'origine que de quantités résiduelles non métabolisés très faibles, voire nulles ;
- d'autre part, n-paraffines et cyclo-paraffines paraissent avoir un comportement identique, seul le nombre d'atomes de carbone intervenant. De plus, il semble qu'il existe une discontinuité au niveau C_{20}-C_{21}; en deçà, les rétentions sont du même ordre de grandeur et le niveau, 15 jours après l'ingestion, se situe autour de 8 p.100 de la dose ingérée ; au-delà de cette limite, la rétention décroît jusqu'à devenir nulle(cas du dotriacontane).

Les résultats concernant la rétention nous amènent à constater qu'après une diminution assez rapide durant les trois premiers jours, les quantités résiduelles retenues dans la carcasse entière atteignent un palier entre le 5ème et le 21ème jour, compte tenu de l a variabilité individuelle. Ceci nous a conduit naturellement à envisager une accumulation chez l'animal à la suite d'une administration prolongée.

II. Accumulation et localisation tissulaire

- Une expérience préliminaire consistant à répéter l'administration de 15 mg d'heptadécane et de dodecylcyclohexane durant 7 jours consécutifs a conduit à des résultats comparables, à savoir l'accumulation dans la carcasse d'environ 7 p.100 de la dose ingérée, signifiant que durant cette période l'accumulation est linéaire. On observe une fixation préférentielle au niveau des graisses de réserve (300 ppm) et du muscle (35 ppm). Pour ce qui est des organes prélevés, les teneurs se situent entre 3 et 20 ppm pour foie, coeur, poumon, rate ou rein.

- Une expérience d'accumulation à long terme a été réalisée dans le cadre d'un ensemble d'études concernant la toxicologie des algues spirulines en collaboration avec le Laboratoire de Toxicologie de la Faculté de Pharmacie. Il s'est avéré que ces algues renfermaient 0,2 p.100 d'hydrocarbures dont les deux tiers sont constitués par de l'heptadécane. La rétention de cette paraffine a été étudiée chez le Rat recevant un régime contenant 25 p.100 d'algues spirulines. La rétention dans les carcasses a été mesurée tous les mois pendant 12 mois et à la fin de cette expérience les teneurs dans les divers organes ont été déterminées.

Les résultats sont les suivants :

- La quantité résiduelle d'heptadécane dans les carcasses plafonne dès le 4ème mois ; elle atteint en moyenne 17 mg chez la femelle et 33 mg chez le mâle, ce qui correspond quel que soit le sexe à environ 65 ppm par rapport au poids vif à l'abattage.

- Au niveau des organes et tissus, les teneurs les plus élevées sont indiquées ci-dessous :

	♂	♀
Tissus adipeux	80 ppm	270 ppm
Muscle	11 "	10 "
Foie	2,5 "	2 "

Des quantités résiduelles de l'ordre de 2 à 15 µg sont relevées dans le cerveau, le coeur, les poumons, la rate et les reins chez les mâles comme les femelles.

- Ces premiers résultats nous ont conduit à envisager une expérience à long terme réalisée dans les conditions suivantes :

. administration ad libitum à des rats mâles d'environ 200 g d'un aliment dans lequel l'hydrocarbure étudié est incorporé à la dose de 0,1 p.100 pendant 6 mois à moins que les quantités résiduelles n'atteignent un plateau au niveau de la carcasse. Les quantités ingérées sont mesurées quotidiennement et des groupes de 3 animaux sont abattus périodiquement ;

. à l'obtention du plateau éventuel ou au bout des 6 mois, les animaux restants sont divisés en deux lots. L'un reçoit une alimentation restreinte de façon à suivre l'évolution de la quantité stockée au cours de l'utilisation des réserves lipidiques. Cette évolution est comparée à celle observée dans l'autre lot qui reçoit ad libitum le régime initial mais sans hydrocarbure.

Ce protocole a été utilisé pour deux hydrocarbures qui donnaient lieu à une rétention importante à la suite d'une administration unique : le dodecylcyclohexane et l'eicosane.

- Les chiffres correspondant aux quantités résiduelles dans les carcasses nous invitent à conclure à une différence de comportement des deux produits étudiés :

. pour le dodecylcyclohexane, il semble que l'on atteigne un maximum voisin de 130 mg dès le 2ème mois ;

. en revanche, les 370 mg d'eicosane accumulés ne peuvent être considérés comme un maximum même si la teneur dans le tissu adipeux semble s'être stabilisée.

- Un point commun est constitué par le fait que l'accumulation s'est effectuée de façon quasi linéaire et que les teneurs finales observées tant au niveau du muscle que du tissu adipeux sont voisines pour les deux hydrocarbures.

- Il est difficile de comparer les résultats obtenus lors de l'amaigrissement du fait des différences relevées dans les quantités stockées et dans les poids des animaux en fin d'accumulation, mais nous pouvons noter qu'une utilisation partielle des réserves lipidiques entraîne une mobilisation d'abord lente des hydrocarbures stockés se traduisant par un accroissement de la teneur dansle tissu adipeux. Puis lorsque toutes les réserves ont été utilisées, seule subsiste une faible quantité résiduelle correspondant sans doute à celle fixée au niveau des lipides structuraux.

- Soulignons encore le fait que dans le cas du dodecylcyclohexane seule la moitié de la quantité stockée est métabolisée en 5 mois lorsqu'un régime normal est distribué ad libitum aux animaux.

- En ce qui concerne les quantités résiduelles observées au niveau des divers organes, elles sont très voisines en fin d'accumulation pour les deux hydrocarbures. Nous en retrouvons environ 130 µg dans les poumons et les reins alors que le cerveau, la rate et le coeur n'en contiennent que de 10 à 30 µg. En revanche, pour les rats amaigris comme pour ceux qui recevaient ad libitum une alimentation normale, les organes n'en contiennent que de 3 à 15 µg.

Conclusion

Les hydrocarbures envisagés sont très largement absorbés tant que le nombre d'atomes de carbone n'est pas trop élevé; en fonction de leur nature, ils donnent lieu à une accumulation plus ou moins importante dans les carcasses et dans tous les cas, le tissu adipeux est le lieu de fixation préférentiel. Enfin, lorsqu'on cesse l'administration, la quantité stockée est mobilisée lentement même si les animaux sont soumisà un régime lipidoprive.

DISCUSSION

MERIAN (Suisse)

Vous avez étudié les mécanismes metaboliques après administration orale des hydrocarbures. Puisqu'on trouve environ 0,5 à 1 mg d'hydrocarbures par m^3 dans l'atmosphère des rues, l'inhalation joue aussi un rôle important. 80% des hydrocarbures dans l'air sont des paraffines 20% des aromates. Est-ce que vous voulez faire des commentaires en relation à cette inhalation qui est principalement une inhalation d'essence.

TULLIEZ (France)

Je dirai tout d'abord que le chiffre de 80% de paraffines parmi les hydrocarbures présents dans l'atmosphère me surprend quelque peu. Toutefois, il est certain que cette présence permanente de paraffines dans l'air devait susciter l'étude de leur absorption par la voie pulmonaire et ce d'autant plus que même lors de l'administration per os de n-alcanes ou cycloalcanes à chaîne longue, le poumon est l'un des sites de fixation préférentielle (nettement après le tissu adipeux et le muscle cependant); or il a été décrit des cas de pneumopathies huileuses et d'oléogranulome pulmonaire chez l'Homme.

DISTRIBUTION IN THE BODY OF THE FLUORIDE INTRODUCED FROM THE DIET IN HIGH DOSES AND ITS PLACENTA TRANSFER

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ABSTRACT

Rabbits were fed on a controlled diet containing different amounts of fluoride (1ppm and 60ppm of F^- in the water).

After a certain period of this treatment the rabbits were mated and, after the birth of the baby rabbits, were killed together with the new-borns. The following parameters were controlled during the course of the experiment which lasted 100 days.

- *a) daily excretion of the fluoride with the urine;*
- *b) daily elimination of the fluoride with the faeces;*
- *c) dosage of the fluoride in the blood;*
- *d) dosage of the fluoride in the bones, teeth, muscles and principal soft tissues of the rabbits at the end of the experiment;*
- *e) dosage of the fluoride in the new-born rabbits and in the placentas.*

The data was compared with the results obtained by similar researches on non-pregnant rabbits (controls).

From all researches carried out the Authors conclude that:

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- Fluoride introduced from the diet is partly eliminated with the faeces and partly absorbed; a very small part of the absorbed fluoride is found in the blood, while a large amount, proportional to that introduced, is excreted with the urine.

- The hard tissues (teeth and bones) fix notable amounts of fluoride in proportion to the quantity introduced, while in the soft tissues the amount of fluoride is always small.

- During the pregnancy the rabbits, especially those treated with high concentrations of fluoride, eliminate a higher quantity of the halogen with the urine than the control rabbits, while a smaller quantity is fixed in the hard and soft tissues.

- The placenta allows the transfer of the fluoride to the foetus in an almost constant quantity; in fact the quantity of fluoride found in the new-born rabbits is always the same, independent of their weight and the quantity of fluoride taken by the mother. The placenta itself in part fixes the fluoride, especially when the amount introduced is very high.

In rabbits fed on diets containing different amounts of fluoride, during the pregnancy, therefore, a part of the halogen is subtracted from the incorporation into maternal tissues, passing through the placenta to the foetus; the urine excretion and the placenta are the regulating organs of an excessive passage of the fluoride to the foetus. This is also demonstrated by the rarity of mottled enamel of deciduous teeth in endemic fluorosis areas.

1. Introduction

It is well known that the biological action of fluoride is different depending on its concentration; in fact while it has been shown that an excess of fluoride introduced into the body may represent a danger owing to its high toxicity, it is also certain that an insufficient introduction of this element affects the mineralisation process of the biological hard tissues (bone and teeth) and influence the formation of dental caries.

Several papers have been published directed to study the distribution in the body of the fluoride introduced in optimum doses by the digestive tract and its passage from the mother to the foetus, in order to contribute to the study of dental caries prophylaxis by the fluoride.

Less numerous instead are the researches directed to investigate the placenta transfer when the fluoride is introduced in high doses.

Smith and Smith [1], Mc Clure[2], Marci[3], have emphasized the rarity of mottled enamel in deciduous teeth in endemic fluorosis areas and have connected this fact to the limited permeability of the human placenta to a certain amount of fluoride (Gardner et al.[4], Feltman and Kosel[5], Ziegler [6], Ericsson and Ullberg [7], Auermann et al.[8], Gedalia et al. [9], Gedalia et al.[10], Blayney and Hill [11],Ericsson and Hammarström [12], Gedalia et al.[13], Gedalia [14]) and also of the placenta of laboratory animals, such as rabbits and rats (Murray [15], Maplesden et al.[16]).

2. Experimental

To study the distribution of the fluoride in the body and its placenta transfer we used rabbits, because the placenta of these animals is classified under group IV of Grosser's classification, that is haemocorial as human placenta (Stefanelli [17]).

The research was carried out on 20 primiparous rabbits aged 4 months and weighing about 2 Kg, kept in metabolic cages throughout the treatment period, in order to collect daily separately the faeces and urine.

To all the animals food and water containing two different amounts of fluoride were administered in order to study the different distribution in the body of the halogen introduced from the diet in optimum and in high doses, normally considered toxic.

The 20 rabbits were divided into two groups: the first group was kept as a control and therefore not mated, while the second group was mated.

For further details of this research, we refer to our works published in detail (Seppilli et al.[18], Scassellati Sforzolini et al.[19]).

2.1 Distribution of the fluoride in the body of control rabbits (Group I)

During the first two weeks of the experiment the 10 rabbits were fed daily on 100 g of food (containing 20.203 ppm F^-) and non-fluoridated water and in the daily faeces and urine the fluoride present was determined (Tab. I). The rabbits were successively subdivided into two sub-groups a) and b): the rabbits of the sub-group I-a) were fed daily on 100 g of food and water containing optimum doses of fluoride (1 ppm of F^-); the rabbits of sub-group I-b) were fed daily on 100 g of food and water containing high doses of fluoride (60 ppm of F^-).

In the first two weeks of this second phase of the experiment in the faeces and urine the fluoride was determined daily (Tab. II), while for the rest of the experimental period which lasted in all 100 days, the fluoride was measured once a week only in the urine (Fig. 1). In the same rabbits the fluoride present in the blood was also determined (Tab. III).

At the end of the experimental period, all the rabbits were killed and in the hard and soft tissues the fluoride present was measured (Tab. IV and Fig. 2).

From the results given in the tables and in the figures we can emphasize that:

1) The amount of fluoride eliminated with the faeces is connected with the means of its administration; in fact the highest percentage of the halogen is eliminated when it is introduced only through the food.

2) The urinary excretion of the fluoride in both the sub-groups remains relatively constant during the whole period of treatment and the amount found is proportional to the quantities introduced.

3) The quantities of the fluoride found in the blood are constant in time and always very low, even if in proportion to the quantities introduced.

4) The tissues wich most concentrate the fluoride are the hard tissues (teeth and bones), while in the soft tissues the amounts found are always small; the amount of fluoride fixed by the various tissues increases with the increase of the amount introduced.

2.2 Distribution in the body of the fluoride during the pregnancy and its placenta transfer (Rabbits Group II).

As in the experiments on the control rabbits, this group was subdivided into two sub-groups: to every rabbit of each sub-group II-a) and II-b) was given food and fluoridated water, containing two different amounts of fluoride: 1 ppm of F^- and 60 ppm of F^-, following the same proceedure described for the control rabbits; the only difference was that the treatment with food and non-fluoridated water lasted only one week.

After 9 weeks from the beginning of the experiment, all the rabbits were mated. For these rabbits also, as in the controls, the treatment lasted a total of 100 days, during which time the urinary excretion was determined once a week (Fig. 3).

At the end of the experiment, both the rabbits and the new-borns (these last immediately after the birth) were killed and the fluoride in the different tissues of the mothers, in the plecentas and in the new-borns "in toto" was determined (Tables VI, VII, VIII).

The results of this second group of researches have been compared with the results of the researches carried out on control rabbits (first group) (Fig. 4).

From the results shown in the tables and in the figures we can emphasize that:

1) During the pregnancy the amount of fluoride excreted with the urine greatly increases in both the sub-groups and in particular in sub-group II-b), and than decreases immediately after the pregnancy to levels more or less normal.

2) The weights of the new-borns and of the placentas of rabbits of the same sub-group don't differ substantially, whereas the new-borns of rabbits from sub-group II-b) constantly weight less than the new-borns of rabbits of sub-group II-a).

3) In the hard and soft tissues of pregnant rabbits the amount of fluoride present is always less than that found in the same tissues of non-pregnant control rabbits.

4) In the new-borns, independent of their weight, fluoride is always found in the same quantity for two sub-groups.

5) In the placentas fluoride is always present and in quantities proportional to the amount introduced.

3. Conclusions

From the researches carried out we can conclude:

- Fluoride introduced from the diet is partly eliminated with the faeces and partly absorbed; a very small part of the absorbed fluoride is found in the blood, while a large amount, proportional to that introduced, is excreted with the urine.

- The hard tissues (teeth and bones) fix notable amounts of fluoride in proportion to the quantity introduced, while in the soft tissues the amount of fluoride is always small.
- During the pregnancy the rabbits, especially those treated with high concentrations of fluoride, eliminate a higher quantity of the halogen with the urine than the control rabbits, while a smaller quantity is fixed in the hard and soft tissues.
- The placenta allows the transfer to the fluoride to the foetus in an almost constant quantity; in fact the quantity of fluoride found in the new-born rabbits is always the same, independent of their weight and the quantity of fluoride taken by the mother. The placenta itself in part fixes the fluoride, especially when the amount introduced is very high.

In rabbits fed on diets containing different amounts of fluoride, during the pregnancy, therefore, a part of the halogen is subtracted from the incorporation into maternal tissues, passing through the placenta to the foetus; the urine excretion and the placenta are the regulating organs of an excessive passage of the fluoride to the foetus. This is also demonstrated by the rarity of mottled enamel of deciduous teeth in endemic fluorosis areas.

Table I

μg of F^- eliminated daily with the faeces and urine of control rabbits fed on diet containing fluoride and non-fluoridated water. Average from 10 rabbits with standard deviation (σ) and variation coefficient (Cv%). Determinations carried out for two weeks: weekly averages with test of significance of the difference between the two averages (t and P).

Days from beginning of treatment	Rabbits fed on 2.0203 mg of F^- daily (only with the diet)					
	Faeces			Urine		
	μg F^- eliminated daily (average from 10 rabbits)	σ	Cv%	μg F^- excreted daily (average from 10 rabbits)	σ	Cv%
2nd	1226.4	53.5884	4.3695	330.3	24.3363	7.3679
3rd	1261.8	58.5525	4.6404	304.6	23.3445	7.6639
4th	1151.7	55.5822	4.8261	328.9	26.4354	8.0375
5th	1252.1	59.4675	4.7494	290.4	23.4153	8.0631
6th	1230.5	54.9675	4.4670	340.3	25.6131	7.5266
7th	1167.6	56.4998	4.8385	298.8	23.5635	7.8860
Average of 1st week	1215.0			315.6		
8th	1144.5	52.7445	4.6085	320.2	24.1833	7.5525
10th	1175.7	47.4654	4.0372	297.4	21.8184	7.3364
12th	1214.3	52.7565	4.3446	312.6	20.4822	6.5522
14th	1190.2	48.7644	4.0971	316.5	23.8839	7.5462
Average of 2nd week	1181.3			311.8		
t	1.3124			0.3509		
P	>0.05			>0.05		

Table II

µg of F⁻ eliminated daily with the faeces and urine of control rabbits (group I) fed on diet containing fluoride and water containing different amounts of fluoride: sub-group I-a) = 1 ppm of F⁻; sub-group I-b) = 60 ppm od F⁻. Average from 5 rabbits per sub-group with standard deviation (σ) and variation coefficient (C_v%). Determinations carried out for two weeks: weekly averages with test of significance of the difference between the two averages (t and P).

Days from beginning of treatment	Sub-group I-a): rabbits fed on 2.0553 mg of F⁻ daily (2.0203 mg with the diet and 0.035 mg with the water)						Sub-group I-b): rabbits fed on 4.0203 mg of F⁻ daily (2.0203 mg with the diet and 2 mg with the water)					
	Faeces			Urine			Faeces			Urine		
	µg F⁻ eliminated daily (average from 5 rabbits)	σ	C_v%	µg F⁻ excreted daily (average from 5 rabbits)	σ	C_v%	µg F⁻ eliminated daily (average from 5 rabbits)	σ	C_v%	µg F⁻ excreted daily (average from 5 rabbits)	σ	C_v%
2nd	1230.6	52.6496	4.2783	363.5	25.7127	7.0736	2045.0	63.1485	3.0879	645.1	45.8933	7.1141
3rd	1240.5	54.2095	4.3699	352.4	25.9347	7.3594	2026.0	63.3597	3.1273	755.2	47.7944	6.3287
4th	1231.2	52.7350	4.2832	325.4	25.1654	7.7336	2100.5	65.9239	3.1384	802.4	52.5794	6.5527
5th	1246.6	50.4291	4.0453	380.6	25.3450	6.6592	2000.4	66.1231	3.3054	704.6	43.5966	6.1874
6th	1215.8	50.6859	4.1689	371.5	25.8737	6.9646	2020.8	65.8506	3.2586	821.3	49.2923	6.0017
7th	1204.5	54.0900	4.4906	318.6	25.4588	7.9908	2030.4	66.0425	3.2526	746.2	46.0375	6.1695
Average of 1st week	1228.2			352.0			2037.2			745.8		
8th	1200.4	50.1499	4.1777	327.3	26.3110	8.0388	2022.4	65.6656	3.2469	781.8	46.7336	6.2335
10th	1170.8	50.9904	4.3551	394.0	25.1319	6.3786	1980.6	63.5744	3.2098	750.0	50.9177	6.7890
12th	1205.2	50.6277	4.2007	345.6	25.6729	7.4285	2010.0	63.9507	3.1816	791.6	49.6070	6.2662
14th	1217.3	55.6316	4.5700	326.4	25.3311	7.7607	1990.2	63.9352	3.2125	800.6	45.9005	5.7332
Average of 2nd week	1198.4			348.3			2000.8			781.0		
t	2.6695			0.2050			1.9147			1.0345		
P	>0.05			>0.05			>0.05			>0.05		

Table III

ppm of F^- in the blood of control rabbits (group I) fed on diet containing fluoride and water containing different amounts of fluoride: sub-group I-a) = 1 ppm of F^-; sub-group I-b) = 60 ppm of F^-. Average from 5 rabbits per sub-group with standard deviation (σ) and variation coefficient ($C_v\%$).

Hours from beginning of treatment	Sub-group I-a): rabbits fed on 2.0553 mg of F^- daily (2.0203 mg with the diet and 0.035 mg with the water)			Sub-group I-b): rabbits fed on 4.0203 mg of F^- daily (2.0203 mg with the diet and 2 mg with the water)		
	ppm of F^- in the blood (average from 5 rabbits)	σ	$C_v\%$	ppm of F^- in the blood (average from 5 rabbits)	σ	$C_v\%$
1	0.18	0.0192	10.6667	1.25	0.1115	8.9200
3	0.15	0.0186	12.4000	1.40	0.1080	7.7143
6	0.14	0.0174	12.4285	1.25	0.1100	8.8000
10	0.16	0.0198	12.3750	1.35	0.1090	8.0741
24	0.17	0.0197	11.5882	1.10	0.1120	10.1818
48	0.13	0.0168	12.9231	1.60	0.1164	7.2750
72	0.19	0.0203	10.6842	1.10	0.1065	9.6818
96	0.18	0.0199	11.0555	1.60	0.1128	7.0500
120	0.15	0.0190	12.6666	1.10	0.1080	9.8182
144	0.16	0.0200	12.5000	1.44	0.1057	7.3437
168	0.15	0.0179	11.9333	1.25	0.1100	8.8000
Average	0.16			1.31		

Table IV

mg of F^- in the hard and soft tissues of control rabbits (group I) fed for 100 days with diet containing fluoride and water containing different amounts of fluoride: sub-group I-a) = 1 ppm of F^-; sub-group I-b) = 60 ppm of F^-. Average from 5 rabbits per sub-group with standard deviation (σ) (calculated on F^- values in total material) and variation coefficient ($C_v\%$).

Organs	Sub-group I-a): rabbits fed on 2.0553 mg of F^- daily (2.0203 mg with the diet and 0.035 mg with the water)							Sub-group I-b): rabbits fed on 4.0203 mg of F^- daily (2.0203 mg with the diet and 2 mg with the water)						
	Average from 5 rabbits							Average from 5 rabbits						
	Dry weight (in g) of total material	% of ashes over dry weight	ppm of F^- in the ashes	ppm of F^- in dry material	mg of F^- in total material	σ	$C_v\%$	Dry weight (in g) of total material	% of ashes over dry weight	ppm of F^- in the ashes	ppm of F^- in dry material	mg of F^- in total material	σ	$C_v\%$
Bones	113.1624	39.2554	897.4960	347.0145	39.8690	4.3859	11.0008	152.0224	45.4759	1206.6740	548.7461	83.4217	9.8388	11.7940
Teeth	5.9380	81.6100	422.5950	344.8804	2.0475	0.2044	9.9809	4.7734	83.5546	806.3140	673.7126	3.2159	0.3510	10.9145
Internal organs (*)	40.3012	4.8540	55.9730	2.7170	0.1095	0.0157	14.3378	32.0635	5.2780	119.4810	6.3062	0.2022	0.0342	16.9130
Thyroid	0.3999	29.5460	n.d.	n.d.	n.d.	-	. -	0.4877	30.1380	n.d.	n.d.	n.d.	-	-
Muscle	445.0560	3.3101	101.0240	3.3440	1.4983	0.2320	15.4842	349.1742	4.6040	279.9760	12.8901	4.5009	0.6703	14.8925

(*) - lungs, heart, liver, pancreas, spleen, kidneys and genitals.
n.d.- non detectable.

Table V

mg of F⁻ fixed by the various organs of the control rabbits (group I) fed for 100 days with diet containing fluoride and water containing different amounts of fluoride: sub-group I-a) = 1 ppm of F⁻; sub-group I-b) = 60 ppm of F⁻.

Animals	Total mg of F⁻ fixed by the various organs examined	mg of F⁻ found in the various organs of the rabbits							
		Bones	% of total F⁻ fixed	Teeth	% of total F⁻ fixed	Internal organs	% of total F⁻ fixed	Muscle	% of total F⁻ fixed
Sub-group I-a): rabbits fed on 2.0553 mg of F⁻ daily (2.0203 mg with the diet and 0.035 mg with the water)	43.5247	39.8690	91.6009	2.0479	4.7051	0.1095	0.2516	1.4983	3.4424
Sub-group I-b): rabbits fed on 4.0203 mg of F⁻ daily (2.0203 mg with the diet and 2 mg with the water)	91.3407	83.4217	91.3303	3.2159	3.5208	0.2022	0.2214	4.5009	4.9276

Table VI

Weight of newborn rabbits and of the placentas of rabbits (group II) fed for 100 days with diet containing fluoride and water containing different amounts of fluoride: sub-group II-a) = 1 ppm of F⁻; sub-group II-b) = 60 ppm of F⁻.

	N. of rabbits	Fresh wgt (in g) of each newborn					Fresh wgt (in g) of all newborns	Dry wgt (in g) of all newborns	% ashes over dry wgt	Placentas		
		I	II	III	IV	V				Fresh wgt (in g) of all placentas	Dry wgt (in g) of all placentas	% ashes over dry wgt
Sub-group II-a): rabbits fed on 2.055 mg of F⁻ daily(2.020 mg with the diet and 0.035 mg with the water)	1	64.0	60.0	59.0	37.0	-	220.0	46.5920	12.8322	4.9	0.8243	10.0736
	2	56.5	42.5	66.5	60.0	55.0	280.5	66.3400	10.0976	7.3	0.9498	11.2230
	3	57.5	62.3	63.5	64.7	-	228.0	52.4322	13.8742	5.0	0.8444	10.3242
	4	61.4	62.8	70.3	-	-	194.5	40.4851	11.1245	4.1	0.7614	9.0138
	5	49.7	70.4	45.1	46.8	-	212.0	47.0122	13.4990	4.7	0.8021	9.8764
	Average						227.0	50.5723	12.2855	5.2	0.8364	10.3042
Sub-group II-b): rabbits fed on 4.020 mg of F⁻ daily(2.020 mg with the diet and 2 mg with the water)	1	50.0	52.0	43.2	-	-	146.1	28.0565	10.8413	4.8	0.7825	11.0078
	2	71.3	49.8	37.9	66.4	-	225.4	46.8349	14.7510	6.0	0.9830	12.0548
	3	39.8	33.2	30.4	44.3	-	147.7	30.7914	13.8115	5.5	0.9700	14.5984
	4	54.2	44.3	48.7	47.4	39.0	233.6	50.4469	15.1015	7.7	1.2000	13.5882
	5	48.7	39.8	45.9	-	-	134.4	26.2363	12.0842	5.0	0.8205	10.9878
	Average						177.4	36.4732	13.3179	5.8	0.9512	12.4474

Table VII

mg of F^- in the hard and soft tissues of pregnant rabbits (group II) fed for 100 days with diet containing fluoride and water containing different amounts of fluoride: sub-group II-a): = 1 ppm of F^-; sub-group II-b): = 60 ppm of F^-. Average from 5 rabbits per sub-group with standard deviation (σ) (calculated on F^- values in total material) and variation coefficient (C_v%).

Organs	Sub-group II-a): rabbits fed on 2.0553 mg of F^- daily (2.0203 mg with the diet and 0.035 mg with the water)							Sub-group II-b): rabbits fed on 4.0203 mg of F^- daily (2.0203 mg with the diet and 2 mg with the water)						
	Average from 5 rabbits							Average from 5 rabbits						
	Dry weight (in g) of total material	% of ashes over dry weight	ppm of F^- in the ashes	ppm of F^- in dry material	mg of F^- in total material	σ	C_v%	Dry weight (in g) of total material	% of ashes over dry weight	ppm of F^- in the ashes	ppm of F^- in dry material	mg of F^- in total material	σ	C_v%
Bones	125.4000	41.4947	753.8870	312.8238	39.2281	4.3547	11.1010	120.6348	44.3772	1075.1500	477.1227	57.5576	6.8043	11.8217
Teeth	4.1812	80.8046	410.3700	331.6034	1.3865	0.1418	10.2316	5.6044	80.0978	718.9420	576.1901	3.2292	0.3555	11.0065
Internal organs (*)	35.2985	5.1060	52.0800	2.6602	0.0939	0.0364	14.5215	38.7018	5.1920	80.1800	4.1626	0.1611	8.0257	15.9768
Thyroid	0.4301	25.7212	n.d.	n.d.	n.d.	-	-	0.3863	28.3154	n.d.	n.d.	n.d.	-	-
Muscle	379.7241	4.2984	91.1910	3.9197	1.4884	0.2355	15.8244	548.4061	4.8925	192.6900	9.4273	5.1700	0.7760	15.0105

(*) - Lungs, heart, liver, pancreas, spleen, kidneys and genitals.

n.d.- non detectable.

Table VIII

mg of F^- in the newborn rabbits and in the placentas of rabbits (group II) fed for 100 days with diet containing fluoride and water containing different amounts of fluoride: sub-group II-a) = 1 ppm of F^-; sub-group II-b) = 60 ppm of F^-. Average from 5 rabbits per sub-group with standard deviation (σ) (calculated on the values of F^- in the total material) and variation coefficient (C_v%).

Material	Sub-group II-a): rabbits fed on 2.0553 mg of F^- daily (2.0203 mg with the diet and 0.035 mg with the water)							Sub-group II-b): rabbits fed on 4.0203 mg of F^- daily (2.0203 mg with the diet and 2 mg with the water)						
	Average from 5 rabbits							Average from 5 rabbits						
	Dry weight (in g) of total material	% of ashes over dry weight	ppm of F^- in the ashes	ppm of F^- in dry material	mg of F^- in total material	σ	C_v%	Dry weight (in g) of total material	% of ashes over dry weight	ppm of F^- in the ashes	ppm of F^- in dry material	mg of F^- in total material	σ	C_v%
Newborns	50.5723	12.2655	32.9960	4.0557	0.2051	3.2174	15.6870	35.4732	13.3179	43.2944	5.7659	0.2103	3.1873	15.1558
Placentas	0.8364	10.3042	257.5410	26.5423	0.0222	0.3267	14.7180	0.9512	12.4474	1072.6350	133.5155	0.1270	2.0641	16.2531

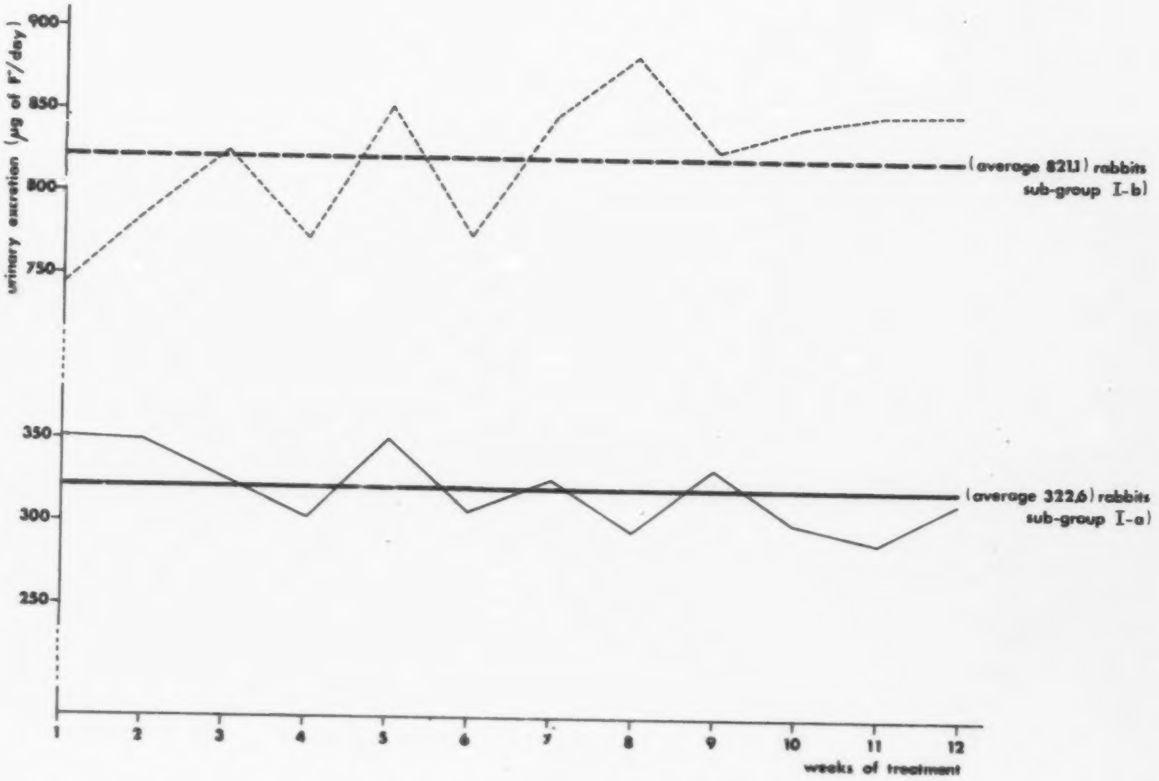


Fig. 1. F$^-$ excreted with the urine of control rabbits (group I) fed on diet containing 20.203 ppm of F$^-$ and water containing different amounts of F$^-$: sub-group I-a) = 1 ppm of F$^-$; sub-group I-b) = 60 ppm of F$^-$.

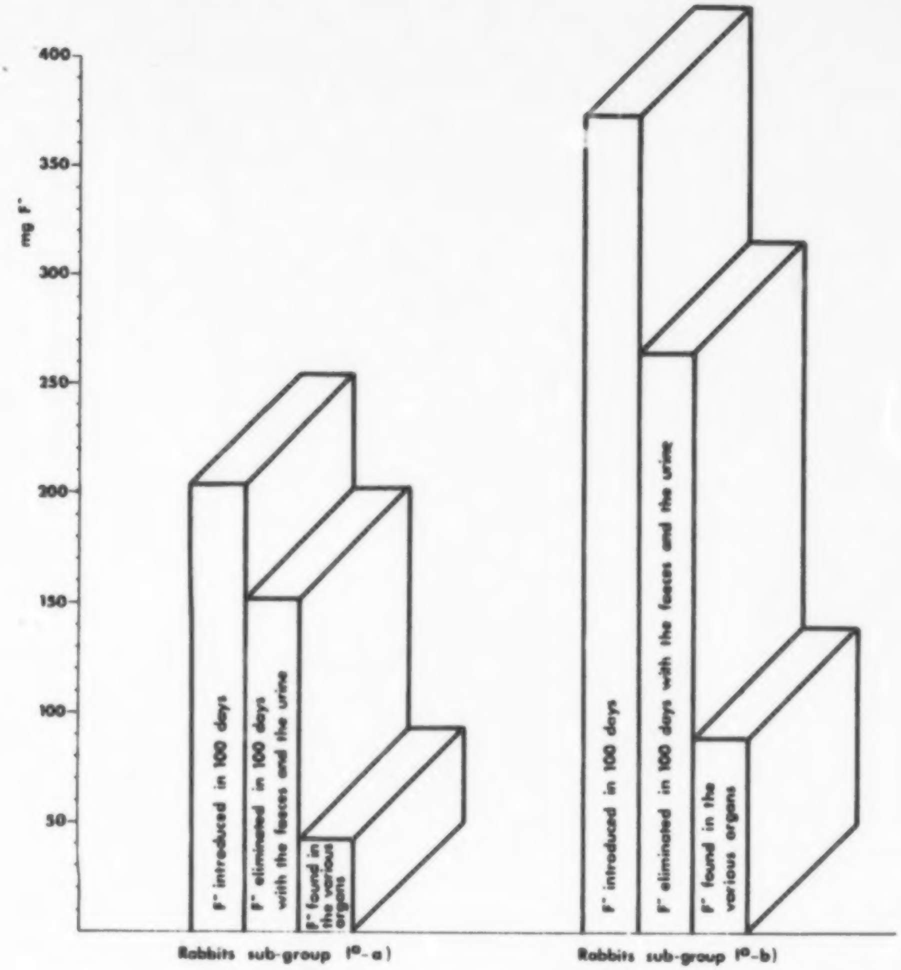
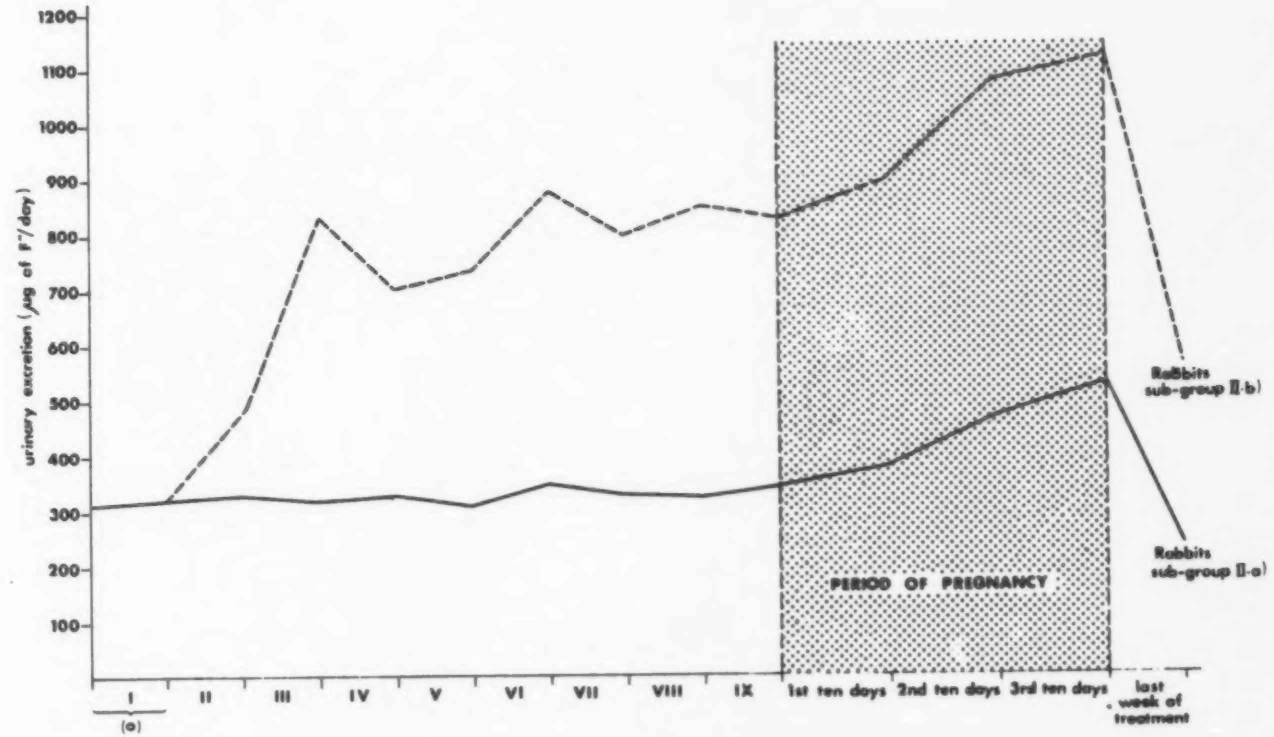


Fig. 2. Distribution of the fluoride in control rabbits (group I) fed for 100 days on diet containing 20.203 ppm of F^- and water containing different amounts of F^-: sub-group I-a) = 1 ppm of F^-; sub-group I-b) = 60 ppm of F^-.



(a) treatment with the diet and non-fluoridated water

Fig. 3. F^- excreted with the urihe of pregnant rabbits (group II) fed on diet containing 20.203 ppm of F^- and water containing different amounts of F^-: sub-group II-a) = 1 ppm of F^-; sub-group II-b) = 60 ppm of F^-.

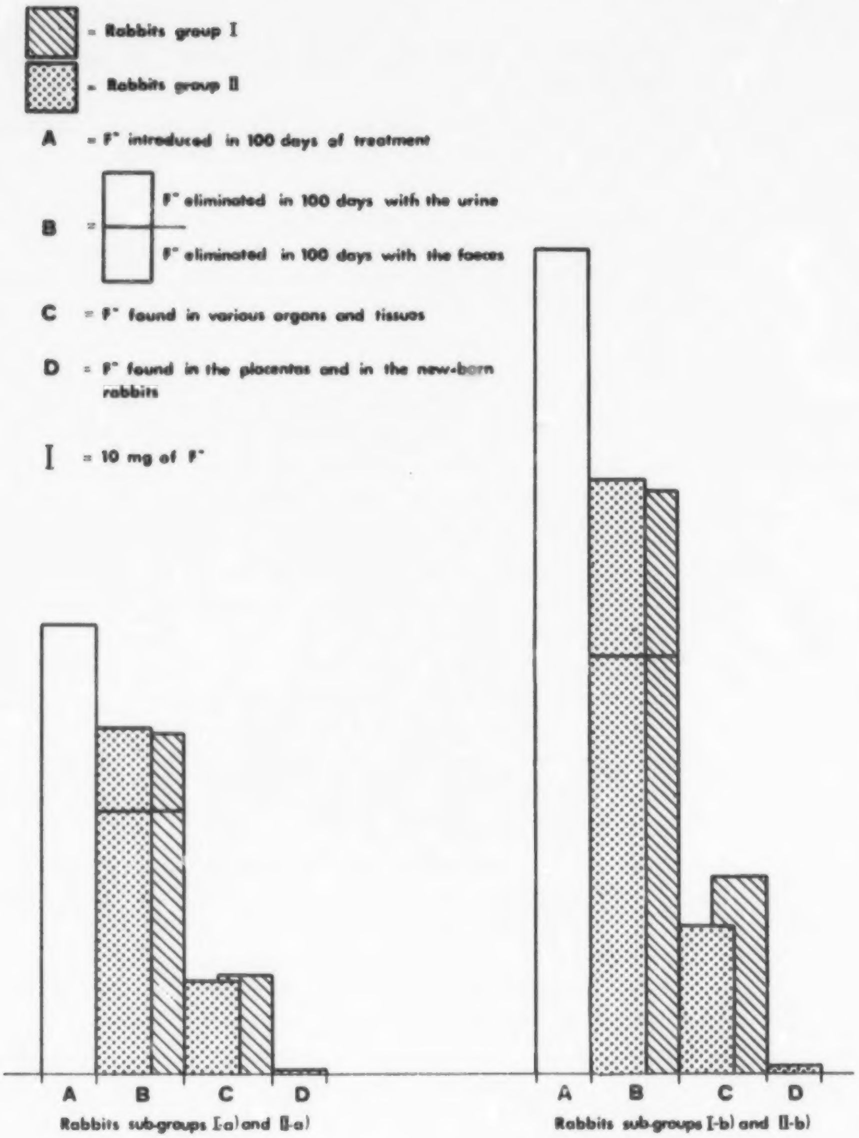


Fig. 4. Distribution of the fluoride in control rabbits (group I) and in pregnant rabbits (group II) fed for 100 days on diet containing 20.203 ppm of F^- and water containing different amounts of F^-: sub-groups I-a) and II-a) = 1 ppm of F^-; sub-groups I-b) and II-b) = 60 ppm of F^-.

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WECHSELBEZIEHUNGEN

INTERACTIONS

INTERAZIONE

INTERACTIES

nder - Chairman - Président - Presidente - Voorzitter.

J. CARSTENSEN (Denmark)

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CADMIUM-ZINC INTERACTIONS

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ABSTRACT

Cadmium accumulates with age in the human kidney. "Normal" exposure causes cadmium concentrations of less than 75 µg/g wet weight in renal cortex at age 50. This "normal" accumulation is accompanied by an equimolar increase in zinc. It has been estimated that at a cadmium concentration of about 200 µg/g wet weight in renal cortex tubular function will be disturbed. The mechanism behind this tubular dysfunction is thought the replacement of zinc by cadmium in certain renal enzymes. There is, however, a lack of data on zinc in relation to cadmium concentrations from 75 to 200 µg/g wet weight. Animal experiments have shown that exposure to cadmium will cause an increase in renal zinc, but exposure has generally been quite high and during short periods of time. Normal horses accumulate cadmium during a life-time, and thus constitute a suitable material for studies of long-term exposure to small amounts of cadmium. Cadmium and zinc were analyzed by atomic absorption spectrophotometry in renal cortex from 37 horses. Cadmium concentrations varied from 5 to 250 µg/g wet weight. At lower concentrations, i.e. < 60 µg/g wet weight, the increase in zinc was equimolar to the increase of cadmium. However, with increasing concentrations of cadmium, zinc did not increase to the same extent, thus indicating a disturbance in the cadmium-zinc relationship. It can be suggested that cadmium may exert an influence on zinc enzymes in the kidney at relatively low concentrations, not far from the present "normal" values.

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1. Introduction

Cadmium and zinc have many common physico-chemical properties and occur together in nature. There are, however, large biological differences. Zinc is an essential metal with short biological half-time, and occurs in high concentrations in all body compartments. Cadmium is non-essential, has an extremely long biological half-time, and accumulates in liver and kidney, the latter organ containing about one third of the total body burden. The placenta differentiates effectively between the two metals, so that whereas the newborn has an excess of zinc, cadmium is virtually absent. Cadmium will accumulate with age in the human kidney, and it has been estimated that at a cadmium concentration of about 200 µg/g wet weight in renal cortex, renal tubular dysfunction may occur. In Europe and the U.S. mean concentrations at age 50 are at present about 30 µg/g wet weight, whereas higher levels have been found in Japan. For further information on metabolism and effects of zinc and cadmium see the books by Prasad et al. [1] , and Friberg et al. [2] respectively.

The renal tubular dysfunction caused by cadmium is characterized by the urinary excretion of low-molecular weight proteins - "tubular proteinuria". The mechanism behind this dysfunction is thought to be a replacement of zinc by cadmium in enzymes taking part in reabsorption, and in catabolism of proteins. Vigliani [3] reported that simultaneous administration of zinc to cadmium-exposed rabbits alleviated the renal damage caused by cadmium alone.

Several animal experiments have shown that exposure to cadmium will cause increases in the zinc concentrations in liver and kidney (see for example Bunn and Matrone [4] , Banis et al. [5] , Anke et al. [6] , Roberts et al. [7] , Cousins et al. [8] , Schroeder and Nason [9]). In these experiments large amounts of cadmium were added to the diet and the cadmium intake was of the same magnitude as the zinc intake - the concentration in the diet or in water being 50 mg/kg or more. This increase is due to a redistribution of body stores of zinc, since it was found in a study on calves that the increase in liver and kidney was accompanied by a decrease in zinc concentrations in muscle and bone. Furthermore, there was a decrease in intestinal absorption of zinc (Roberts et al.[7]). Petering et al. [10] found that when the concentration of zinc was <2 mg Zn/kg in the diet, and 2 mg/kg of drinking water, cadmium at relatively low concentration (3.4 mg/kg of drinking water) caused a decrease in the

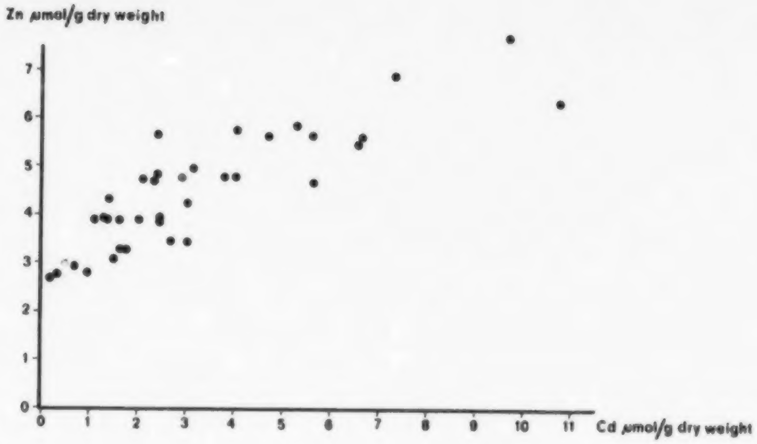

Figure 1. Concentrations of cadmium and zinc in renal cortex from 37 Swedish horses.

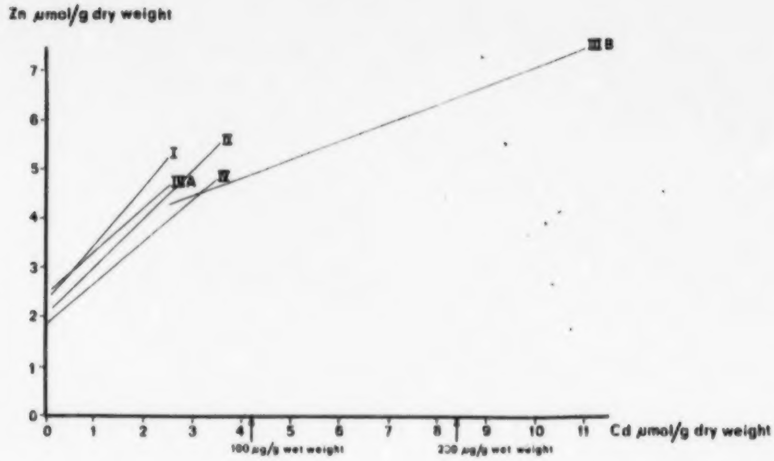

Figure 2. Regression lines for cadmium and zinc in renal cortex.
I Normal human beings (age 6-50 years, n = 36)
from Piscator and Lind [12].
II Normal human beings (age 10-90 years, n = 87)
from Hammer et al. [13].
III Normal horses with A: cadmium concentration below 2.5 µmol/g
(n = 20), and B: above 2.5 µmol/g (n = 17)
IV Experimentally exposed swine (n = 12, whole kidney)
from Cousins et al. [8].

zinc concentrations in rat testes, whereas such a decrease was not noted when the zinc concentration in drinking water was increased to 8 mg/kg.

These results from animal experiments show that cadmium may cause alterations in zinc metabolism and that certain tissues may be depleted of zinc, when cadmium causes an increase of zinc in liver and kidney.

The relationship between cadmium and zinc in organs from human beings without known industrial exposure has been studied using autopsy cases. Schroeder et al. [11] showed that there was a parallel increase in cadmium and zinc levels in kidney with age. Piscator and Lind [12] found the same relationship in renal cortex and could also show that the increase in zinc was equimolar to the increase in cadmium, the regression equation being Zn = 1.13 Cd + 0.5 (μmol/g wet weight). Furthermore, an estimate of the base value for zinc in renal cortex was obtained, being about 34 μg/g wet weight (160 μg/g dry weight). Hammer et al. [13] found that the regression equation was Zn = 0.95 Cd + 29.4 (μmol/g ash), or, transferred to wet weight, Zn = 0.95 Cd + 0.41. However, the cadmium concentrations in these studies have been generally below 75 μg/g wet weight. There was a need for a study of cadmium-zinc relationships at higher cadmium concentrations, i.e. up to 200 μg/g. Normally exposed horses provided the necessary material.

2. Studies on horses

Liver and kidney were obtained from a total of 37 horses, 1-25 years of age, killed at two slaughter-houses, one in the South of Sweden, and one in the North. Cadmium and zinc were determined with atomic absorption spectrophotometry in the same way as for organs from human beings (Piscator and Lind [12]). In figure 1 zinc concentrations are shown in relation to cadmium concentrations in renal cortex. It is seen that at concentrations of cadmium above 3 μmol/g dry weight (about 70 μg/g wet weight), there is not a corresponding increase in zinc as seen at lower concentrations.

In figure 2 the regression lines for the two human studies, the horse study, and one experimental study are shown together. The horse material was divided into two groups with 2.5 μmol/g as border value. It is seen that at low cadmium concentrations the horses do not differ from the human groups, but at higher cadmium concentrations there is no longer an equimolar increase in zinc.

3. Discussion

The results obtained and the results from other investigations suggest that at moderate increases in cadmium concentrations to about 75 µg/g renal cortex (wet weight), there will be an equimolar increase in zinc and the amount of zinc necessary for normal physiological functions is probably adequate. At higher concentrations of cadmium the rate of zinc accumulation will decrease and will no longer be equimolar. The reason for this may be that the cadmium-binding protein, metallothionein, which is the main transport and storage protein for cadmium, contains more cadmium and less zinc than normally, i.e. when there are equimolar amounts of cadmium and zinc. This would mean that some zinc would still be available for enzymatic functions. Another possibility is that an equimolar relationship in metallothionein still exists, in which case less zinc would be available for enzymatic functions in the renal cortex, and hence decreases in enzymatic activities can be expected. That very subtle changes in cadmium-zinc ratios can influence enzymatic activity is suggested by the experimental fundings of Cousins et al. [8], who in swine found that the activity of the zinc enzyme, leucine aminopeptidase, was already decreased at a cadmium concentration of 78 µg/g in whole kidney (corresponding to about 100 µg/g in renal cortex). It is, however, also possible that other metals may have been influenced in this experiment, since high doses were given. Indeed in some of the animals described in the introduction the copper metabolism was also disturbed, which was not the case in the human studies by Piscator and Lind [12].

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DISCUSSION

DIEHL (Federal Republic of Germany)

The great concern about cadmium in the environment goes back primarily to the unfortunate Itat-Itai incident in Japan. A number of authors have already voiced doubts that the symptoms seen there could have been due to cadmium toxicity per se. What we have heard in Dr. Lorke's paper ("Subchronische orale Toxizität von Cadmium bei Ratte und Hund") strengthened the impression that cadmium toxicity is not as high as previously believed. Do you think, on the basis of your studies and of other information you have on Zn/Cd interaction, that the Itai-Itai incident could have been due to a combination of elevated dietary cadmium intake and insufficient zinc intake?

PISCATOR (Sweden)

Since the pollution was caused by a zinc-mine, the levels of zinc in rice were above normal and thus the intake of zinc was sufficient. The calcium intake however, is low in the actual area and the Itai-Itai disease is caused by cadmium acting on a sensitive population with calcium deficiency. Cadmium is highly toxic, the long biological half-time causes an accumulation in the kidney which after many years may reach high levels.

Dr. Lorke's paper stressed what was known before that short-term exposure to relatively low amounts of cadmium does not cause any major dysfunction, but that does not mean that cadmium has a low chronic toxicity.

SCOPPA (Italy)

Have you any idea about the biological half-life of thionein, or metallothioneins?

PISCATOR (Sweden)

No, but the half-times may well be different in liver and kidney, the latter being a catabolic organ.

van der KREEK (Netherlands)

We learned from one of the earlier introductions that cadmium is stored in the thyroid and pituitary glands. You studied the Cd/Zn balance in the kidney. Did you study also the Cd/Zn balance in the thyroid and pituitary glands and the possible influence of excess Cd on the function of thyroid and pituitary glands?

PISCATOR (Sweden)

We have not studied Cd/Zn balance in other organs. In connection with experimental studies on the effects of cadmium on calcium deficient animals also the parathyroids are studied, but not the thyroid or pituitary glands.

HISLOP (U.K.)

Would you care to comment on the distribution of Cd in kidney and why did you chose to analyse the cortex?

PISCATOR (Sweden)

The cadmium concentrations are highest in the cortex and it is easy to standardize the sampling technique.

HEPATIC DAMAGE AND ORGANOCHLORINE RESIDUE CONCENTRATIONS IN BODY TISSUES

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ABSTRACT

Liver specimens, taken from patients with hepatic cirrhosis, contained significantly higher concentrations of DDT, but significantly lower concentrations of heptachlor epoxide, than specimens from controls. Concentrations of dieldrin were not significantly different. No significant differences were found between cirrhotics and controls in the concentrations of any residues in brain and adipose tissues. None of the specimens contained detectable amounts of aldrin, heptachlor, lindane, or the alpha or gamma isomers of chlordane.

There was a highly significant association between lipid contents and residue concentrations in livers. Cirrhotic livers with severe fatty infiltration, determined by histopathological examination, and confirmed analytically, contained significantly higher amounts of DDT and heptachlor epoxide than did those with minimal or no fatty changes.

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1. Introduction

Reportedly, certain diseases, especially those affecting the liver, have been associated with tissue residue concentrations significantly higher than in healthy persons (1). But other workers have found no association between tissue residue concentrations in humans and a variety of pathological conditions (2). The purpose of our studies was to determine whether or not concentrations of organochlorine pesticide residues in tissues of patients with hepatic cirrhosis were higher than in those from controls, and to investigate causes responsible for any differences.

2. Materials and Method

Specimens of liver, brain, and adipose tissue were collected at autopsy, within 48 hours of death, from patients judged clinically to have died of hepatic failure with cirrhosis of the liver. For a control group, an additional series of tissues was collected from patients judged clinically not to have cirrhosis. Residues were extracted from each tissue specimen, identified, and quantified as described in detail by Oloffs *et al*. (3). Lipid concentrations in the liver tissue specimens were measured gravimetrically (3).

For histopathological examination, sections of each liver, fixed in Formol Calcium and stained with haematoxylin and eosin, were examined microscopically for the following: fatty change, fibrosis, central lobular congestion, and bile stasis. Each was ranked on a scale from 0 (= normal) to 5 (= most severe). All slides were examined at the same time in order to assure consistent judgement. The specimens were not identifiable in any way and were presented at random for review. Half of the slides were examined twice in this fashion with consistent results.

3. Results and Discussion

DDT residue concentrations in liver were significantly (*) higher in the specimens from cirrhotics than in those from controls, but heptachlor epoxide concentrations were significantly (**) lower. No statistically significant differences could be demonstrated for residues in adipose and brain tissues. Lipid content of liver specimens was higher (*) in the cirrhotic patients than amoung controls (Table I). Lindane, aldrin, heptachlor, alpha chlordane, and gamma chlordane were not detected in any of the specimens.

Chlorinated hydrocarbon pesticides and some of their metabolites

Table I Mean Residue Concentrations (ppb) of Dieldrin, Heptachlor Epoxide, and DDT in Human Tissues

	CIRRHOTICS		CONTROLS		
	$\bar{x}$	$(n_p;n_o)$[c]	$\bar{x}$	$(n_p;n_o)$[c]	P[a]
		LIVER SPECIMENS			
Dieldrin	8.07	(22;40)	5.98	(21;28)	40
Heptachlor Epoxide	19.28**	(50;12)	34.21	(48;1)	0.1
Total DDT[b]	473.09*	(62;0)	273.64	(48;1)	2
Lipid (% b. w.)	7.97	(62;0)	5.08	(49;0)	5
		ADIPOSE TISSUE			
Dieldrin	69.33	(50;12)	113.66	(38;3)	20
Heptachlor Epoxide	91.45	(59;3)	109.31	(39;2)	20
Total DDT[b]	3681.48	(62;0)	4248.38	(41;0)	50
		BRAIN TISSUE			
Dieldrin	2.00	(0;59)	2.32	(1;20)	-
Heptachlor Epoxide	2.29	(4;55)	2.30	(1;20)	-
Total DDT[b]	34.92	(59;0)	46.52	(21;0)	40

[a] % probability that two means belong to the same population; difference considered significant (*) where $P \leq 5$, highly significant (**) where $P \leq 1$. [b] DDT + DDE + DDD [c] n_p = number of specimens with concentration above, n_o = number of specimens with concentrations below lower limit of quantification; thus $n_p + n_o$ = total number of specimens obtained and analysed.

are highly lipophilic, and they tend to partition into fatty tissue and become concentrated therein. The much higher concentrations in adipose than in liver tissue (Table I) demonstrate this clearly. Furthermore, since lipid content was significantly higher in cirrhotic than in control livers, the question arose to what degree the residue levels in liver tissue may depend on, and vary with, the lipid content, i. e., differences between residues in cirrhotic and control livers may be due to the respective lipid contents, rather than to other liver pathology. Linear correlation coefficients indeed indicated a positive correlation between hepatic lipid and residue concentrations, rather than between liver pathology and residue concentrations. In no case was the correlation negative.

Analysis of the liver data from the cirrhotic patients, grouped according to tissue injury determined by histopathological examination, further supports this view. Heptachlor epoxide, total DDT, and lipid concentrations were significantly (**) higher in livers with moderately severe and severe fatty infiltration, thus confirming the demonstrated association between lipid content, as determined gravimetrically in the laboratory, and residue concentrations. Grouped according to degree of fibrosis, however, the differences were reversed. Both DDT and lipid contents were significantly (*) lower in the group with severe fibrosis, because connective tissue had displaced lipid and thus diminished the reservoir for the lipophilic DDT residues.

4. Conclusion

We have demonstrated that residue concentrations in livers vary strongly and closely with lipid content, regardless of the pathology. Where hepatic cirrhosis was associated with severe fatty infiltration, the residues were higher than those in the controls or in cirrhotics having low liver lipid concentrations. In association with severe fibrosis or bile stasis, the residue concentrations were below those in the controls. If altered hepatic function, rather than liver lipid content, were a major factor contributing to acquisition of pesticide residues in body tissues, differences should have been found also between residue concentrations in adipose and brain tissue from the cirrhosis group and concentrations in the same tissues from the control group. We conclude that lipid content in the liver was the prime determinant of pesticide residue concentration.

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INFLUENCE DE CERTAINES DROGUES SUR L'EFFET TOXIQUE DECLENCHE PAR L'AFLATOXINE B_1 DANS LE FOIE

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ABSTRACT

The effects of a pretreatment of animals with phenobarbital on the inhibition induced by aflatoxin B_1 *on RNA synthesis in liver have been studied in rats and mice.*

Rats are relatively susceptible to aflatoxin action. Pretreatment with phenobarbital decreases the inhibition of transcription due to aflatoxin administration.

Mice are much more resistant than rats to toxic and carcinogenic effects of aflatoxin B_1*. However, pretreatment of animals with phenobarbital, enhances the inhibition of RNA synthesis induced by aflatoxin in liver. Thus, phenobarbital may potentiate the toxic effect of drugs in certain animal species. Implications of these results are discussed.*

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L'aflatoxine B_1, mycotoxine secrétée par Aspergillus flavus, est le plus puissant des hépatocancérogènes connus à ce jour. Elle suscite un intérêt particulier en raison de sa présence possible comme contaminant des aliments de l'homme et de certaines espèces animales ; c'est un danger potentiel sérieux qui doit être pris en considération [voir la revue de WOGAN (1)].

En dehors de son action cancérogène qui se manifeste toujours après un temps de latence relativement long, l'aflatoxine B_1 déclenche à court terme des effets toxiques dans le foie. Ces effets se traduisent sur le plan morphologique par des altérations nucléaires et cytoplasmiques. Sur le plan biochimique, les déviations métaboliques induites par l'aflatoxine touchent surtout la synthèse des acides nucléiques (RNA et DNA) et des protéines. L'inhibition de la synthèse de RNA correspond à un blocage du mécanisme de transcription comme le prouvent les diminutions d'activité RNA polymérase des noyaux de foie isolés à partir d'animaux traités par la mycotoxine.

L'aflatoxine est métabolisée par des systèmes enzymatiques responsables de la transformation des drogues, systèmes qui sont localisés dans les membranes du réseau endoplasmique. Il a été montré que la molécule biologiquement active n'est pas l'aflatoxine elle-même mais un de ses métabolites formés au cours de sa métabolisation [MOULE et FRAYSSINET (2)].

Le traitement des animaux par certains composés, tels les barbituriques, est connu pour induire la synthèse d'enzymes intervenant dans la métabolisation des drogues par l'intermédiaire du cytochrome P_{450}. Le présent travail concerne l'action d'un traitement préalable par le phénobarbital sur les propriétés toxiques de l'aflatoxine B_1 chez deux espèces qui présentent de façon constitutive une grande différence de sensibilité à ses effets : le rat et la souris. C'est ainsi que pour le rat, la DL_{50} oscille entre 1 et 7 mg/kg selon les souches de rat alors que pour la souris, elle se situe aux environs de 60 mg/kg. Ces différences de sensibilité se reflètent sur le plan métabolique : une dose de 1 mg/kg d'aflatoxine B_1 donnée au rat Wistar (souche Commentry) inhibe la synthèse des RNA nucléaires de 80 % (temps d'action : 3 heures) alors que dans les mêmes conditions, une administration de 60 mg/kg à la souris Swiss ne déclenche qu'une inhibition de 40 %. Des résultats du même ordre sont enregistrés au niveau de l'activité RNA-polymérase des noyaux isolés à partir des foies de ces deux espèces (Tableau I).

Le traitement préalable des rats au phénobarbital a toujours comme résultat de diminuer l'inhibition produite par l'aflatoxine B_1 sur la transcription évaluée, soit par l'incorporation in vivo de précurseurs dans le RNA nucléaire, soit par la mesure in vitro de l'activité RNA polymérase des noyaux isolés (Tableau I). La stimulation des systèmes de métabolisation avant l'administration d'aflatoxine B_1 aboutit donc à une diminution des effets toxiques produits par la toxine. Ces résultats confirment les observations de GUMBMANN et WILLIAMS (3), Mac LEAN et MARSHALL (4) et NEAL (5) concernant les variations des pouvoirs toxique et cancérogène de l'aflatoxine B_1 après action des barbituriques.

Les résultats obtenus avec la souris sont totalement différents. Le traitement préalable des animaux par le phénobarbital produit dans la plupart des cas une augmentation de l'inhibition de la transcription induite par l'aflatoxine B_1 (Tableau I). Ainsi par un tel traitement, on peut sensibiliser une espèce animale normalement résistante aux effets toxiques de certains composés. Des observations analogues avaient été faites pour le tétrachlorure de carbone [GARNER et Mac LEAN (6)], le 1,1,1-trichloréthane [CARLSON (7)] et la monocrotaline [ALLEN et al. (8)] chez le rat et pour la rétrorsine chez le cobaye [WHITE et al. (9)]; cependant, c'est la première fois, à notre connaissance, que la potentialisation des effets toxiques de l'aflatoxine B_1 par le phénobarbital est signalée.

TABLEAU I

INFLUENCE DU TRAITEMENT AU PHENOBARBITAL SUR L'INHIBITION DE LA TRANSCRIPTION DANS LE FOIE PAR L'AFLATOXINE B_1 CHEZ LE RAT ET LA SOURIS

	% Inhibition *	
	Synthèse *in vivo* de RNA **	Activité RNA polymérase des noyaux isolés
Rats		
- Aflatoxine (1 mg/kg)	83	74
- Phénobarbital puis aflatoxine	35	55
Souris		
- Aflatoxine (60 mg/kg)	41	33
- Phénobarbital puis aflatoxine	63	48

Temps d'action de l'aflatoxine : 3 heures

* Le % d'inhibition est calculé par rapport aux témoins respectifs traités ou non par le phénobarbital.

** Injection d'acide orotique-^{14}C, 30 minutes avant le sacrifice.

L'implication de ces résultats est importante sur le plan de la métabolisation des drogues dont les mécanismes se révèlent extrêmement complexes et dont la stimulation peut aboutir à des situations ambivalentes. D'autre part, elle souligne le danger potentiel que peut présenter la superposition d'action de certaines drogues et, dans ce domaine, on doit évoquer les effets susceptibles d'atteindre l'homme dont le cadre de vie se trouve envahi par la présence de composés capables d'interférer avec son propre métabolisme.

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BRONCHITE CHRONIQUE ET PERTURBATIONS FONCTIONNELLES RESPIRATOIRES EN RELATION AVEC L'EXPOSITION A DIVERSES NUISANCES : ETUDES DANS DIFFERENTS GROUPES SOCIO-PROFESSIONNELS

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RESUME

Une étude épidémiologique a porté sur 581 ouvriers de sexe masculin, âgés de 40 à 60 ans, de différents groupes socio-professionnels habitant la même région de l'Est de la France (ouvriers du bâtiment, ouvriers d'une usine sidérurgique, travailleurs sans exposition professionnelle particulière). Les sujets ont été interrogés à l'aide d'un questionnaire de la bronchite (questionnaire C.E.C.A.), examinés du point de vue clinique et fonctionnel. Les examens fonctionnels suivants ont été pratiqués: détermination de la capacité vitale (CV) du volume expiratoire maximum seconde (VEMS), du volume résiduel (VR), calcul des rapports VEMS/CV, VR/CT; Epreuves au CO, en respiration unique et en régime stable au repos et au cours d'un effort de 40 watts. L'analyse des symptômes de bronchite ainsi que des perturbations fonctionnelles observées a été faite particulièrement en fonction des nuisances professionnelles rencontrées par les ouvriers durant leur travail. L'exposition professionnelle paraît, certes, jouer un rôle non négligeable sur les symptômes de bronchite et sur les perturbations fonctionnelles, mais le rôle des habitudes tabagiques est apparu beaucoup plus important; les nuisances entraînent quelques troubles respiratoires chez les non fumeurs, mais ces troubles sont beaucoup plus fréquents et plus importants

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chez les sujets exposés et gros fumeurs. Il apparaît un effet potentialisateur nuisance et fumée de tabac et ceci est particulièrement net lorsque l'on compare les groupes après standardisation de l'âge.

ABSTRACT

An epidemiological survey was carried out on 581 male workers aged between 40 and 60 from different social and professional groups and living in the same region in Eastern France (building operatives, iron and steel workers, workers with no specific occupational health risks). The test subjects were given a bronchitis questionnaire (ECSC questionnaire) and underwent a clinical and functional examination. The following functional tests were carried out: measurement of the vital capacity (VC) of the maximum volume exhaled per second (MVES) and of the residual volume (RV), calculation of the MVES/VC and RV/TC ratios; tests for CO by single breath and continuous breathing, both at rest and under a 40 watt load. The bronchitis symptoms and the functional disorders that were noted have been analysed with particular regard to the occupational health risks encountered by the workers in their jobs. Indeed, occupational health risks do seem to have a not inconsiderable effect on bronchitis symptoms and functional disorders, but the effects of tobacco smoking habits appeared to be far greater; some respiratory troubles in the non-smokers are the result of the health risks to which they are exposed, but these troubles are much more frequent and more serious in the subjects who are exposed to health risks and are heavy smokers. Health risks seem to be potentiated by tobacco smoke, and this is shown especially clearly when the groups are compared after standardization for age.

L'étude du rôle des nuisances atmosphériques dans le développement des maladies pulmonaires chroniques doit tenir compte de nombreux facteurs, tels que la pollution atmosphérique générale, les variations climatiques, les conditions socio-économiques, mais aussi des expositions professionnelles et des habitudes tabagiques comme le souligne B. FERRIS (4).

Pour étudier plus particulièrement ces deux derniers facteurs, trois groupes socio-professionnels différents habitant la même région, donc soumis aux mêmes ambiances générales mais différents dans leurs expositions professionnelles et dans leurs habitudes tabagiques personnelles ont été examinés. Les résultats observés dans ces trois groupes sont ici présentés et discutés, d'abord en considérant séparément les groupes, puis plus spécifiquement en fonction des expositions.

MATERIEL ET METHODE

Les examens ont été menés dans une ville de l'Est de la France, auprès de trois groupes à peu près égaux de travailleurs. Le premier groupe est constitué d'ouvriers du bâtiment soumis professionnellement aux poussières et aux intempéries, le deuxième groupe est constitué d'ouvriers d'une usine sidérurgique soumis durant leur travail à des polluants multiples (poussières, fumées, gaz irritants), enfin le troisième groupe est formé de travailleurs peu exposés professionnellement, constitué essentiellement par les employés et petits commerçants de l'agglomération.

Seuls les hommes de 40 à 60 ans ont été choisis, dans les deux premiers groupes par tirage au sort sur la liste nominale professionnelle après élimination des sujets ayant une radiographie anormale (séquelles de tuberculose, silicose, abcès du poumon etc ...) et dans le troisième groupe, sur la liste électorale après élimination des sujets pouvant appartenir aux deux groupes précédents. Le tirage au sort est fait en sorte que la répartition soit à peu près identique dans chaque tranche d'âge de 5 ans entre 40 et 60 ans.

Ainsi, 196 ouvriers du bâtiment (groupe I), 200 ouvriers de l'usine sidérurgique (groupe II) et 185 habitants de l'agglomération

FREQUENCE DE LA SYMPTOMATOLOGIE DANS LES 3 GROUPES SOCIO-PROFESSIONNELS

	GROUPE I Ouvriers bât. (196 sujets)		GROUPE II Ouvriers sidérurg. (200 sujets)		GROUPE III Habitants (185 sujets)	
Toux 0 Exp. 0	(100) 51 %		(89) 44,5 %	**	(107) 58 %	
Toux + Exp. 0	(17) 8,5 %		(9) 4,5 %		(4) 2,1 %	*
Toux ± Exp. ±	(38) 19,5 %		(38) 19 %	**	(55) 29,6 %	**
Toux + Exp. +	(41) 21 %	**	(64) 32 %	***	(19) 10,3 %	**
Dyspnée d'effort	(37) 18,9 %		(43) 21,5 %		(39) 31 %	

Tableau I

Les astérisques placés après les chiffres de la 3è colonne indiquent les comparaisons des chiffres de la 1ère colonne et de la 3è, et ceux entre les colonnes, les comparaisons inter-colonnes.

* p < 0,05 ** p < 0,01 *** p <0,001

(groupe III), soit au total 581 sujets, ont été examinés par la même équipe de médecins et techniciens et par les même équipe de médecins et techniciens et par les mêmes appareillages. Un interrogatoire standard de la bronchite chronique est utilisé (interrogatoire adopté par la Communauté Européenne Charbon et Acier (2), dérivé de celui du British Medical Research Council (10)). Il permet de connaître la symptomatologie, les habitudes tabagiques, les expositions professionnelles des sujets. Un examen clinique est ensuite pratiqué, suivi des épreuves fonctionnelles comportant : la détermination de la capacité vitale (CV), du volume expiratoire maximum seconde (VEMS), du volume résiduel (VR) et les calculs des rapports VEMS/CV et VR/CT ; des épreuves au monoxyde de carbone : transfert du CO en apnée, transfert et ductances du CO en régime stable au repos et au cours d'un effort de 40 watts.

RESULTATS

Dans les groupes étudiés, les réponses au questionnaire permettent de dire qu'il y a plus de sujets se plaignant d'une toux et d'une expectoration chroniques depuis au moins 2 ans (répondant ainsi à la définition de la bronchite chronique) chez les ouvriers de l'usine sidérurgique (groupe II) par rapport aux ouvriers du bâtiment (groupe I) et aux habitants de l'agglomération (groupe III). Cette différence est hautement significative ($p < 0,01$ et $p < 0,001$). C'est aussi dans le groupe III qu'il y a le plus de sujets sans symptomatologie. Il n'y a pas de différence quant à la fréquence de la dyspnée d'effort qui est signalée dans les trois groupes à peu près chez 1 sujet sur 5.

Les examens fonctionnels montrent que les valeurs spirographiques sont dans l'ensemble meilleures dans le groupe des ouvriers de sidérurgie (groupe II) par rapport aux deux autres, malgré les données cliniques antérieures. Dans les trois groupes, on note une décroissance de la capacité vitale et du VEMS en % des valeurs théoriques (ces valeurs tiennent compte déjà des facteurs taille et âge des sujets) en fonction de l'âge. Ceci permet de dire que les anomalies spirographiques sont plus fréquentes chez les sujets âgés, en particulier dans le groupe III (Fig. 1 et 2). Il n'existe pas de différence significative dans les épreuves au monoxyde de carbone pour les trois groupes, d'un groupe à

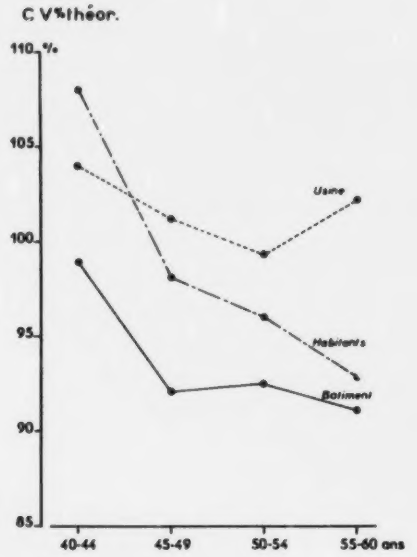

Fig. 1. Modifications de la CV%théorique pour un groupe d'ouvriers de sidérurgie.

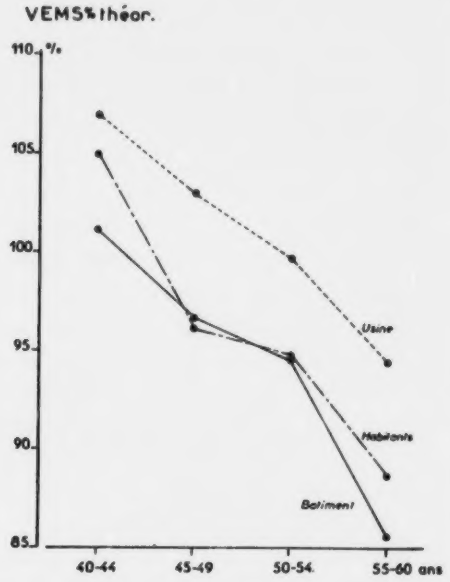

Fig. 2. Modifications du VEMS%-théorique pour un group d'ouvriers de sidérurgie.

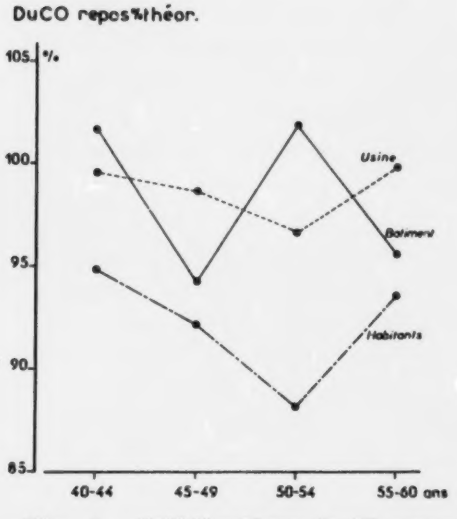

Fig. 3. Modifications du CO repos-%théorique pour un groupe d'ouvriers de sidérurgie.

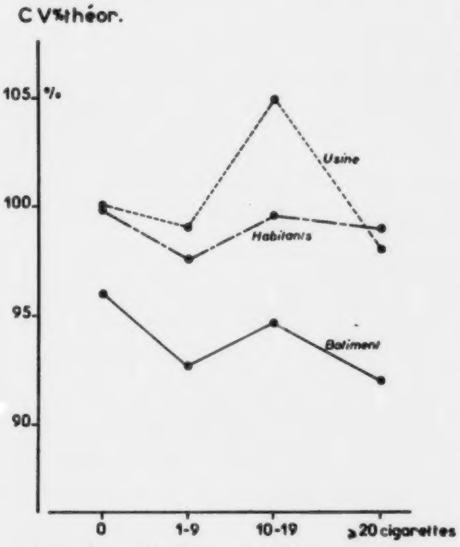

Fig. 4. Effets du tabac sur la CV%théorique.

l'autre et d'une tranche d'âge à l'autre lorsqu'on exprime les résultats en pourcentage des valeurs théoriques (Fig. 3).

En fonction du tabac, on note peu de modifications de la capacité vitale % de théorique (Fig. 4) mais par contre le VEMS % de la théorique est plus abaissé chez les plus gros fumeurs, ceci dans les trois groupes socio-professionnels et laisse penser que l'obstruction bronchique est plus marquée chez ces sujets (Fig. 5). L'efficacité ventilatoire jugée par la ductance du CO est plus abaissée chez les fumeurs, par rapport aux non fumeurs, mais il n'y a pas de différence selon l'importance des habitudes tabagiques (fig. 6).

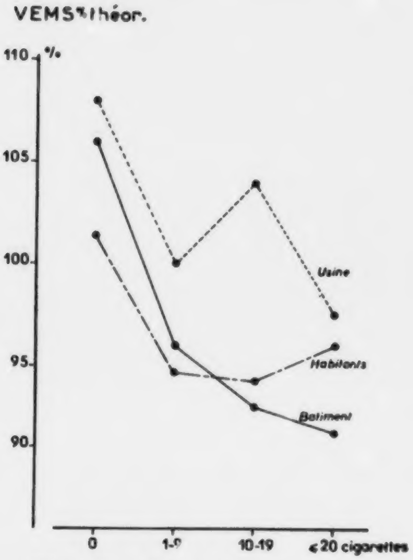

Fig. 5. Effets du tabac sur le VEMS%théorique.

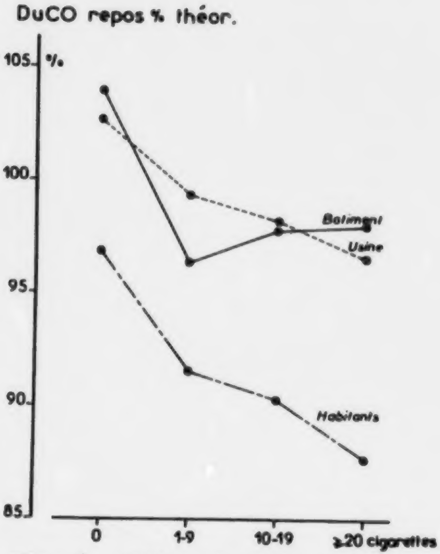

Fig. 6. Effets du tabac sur le CO-repos%théorique.

La différence dans l'exposition tenant compte des groupes socio-professionnels, comme il vient d'être présenté, est relativement grossière. Au sein du même groupe, les sujets sont plus ou moins exposés et il est plus logique de considérer les sujets selon leur exposition individuelle.

L'on constate ainsi que le taux de bronchite est toujours plus élevé chez les sujets ayant une exposition professionnelle, ceci quelle que soit la tranche d'âge envisagée (fig. 7). Cependant l'influence du tabac est aussi indiscutable : chez les non fumeurs, les sujets exposés ont certes un taux de bronchite plus élevé (21 %) que les non exposés (6,5 %), mais ce taux est nettement plus bas que chez les sujets sans exposition professionnelle mais gros fumeurs (39,5 %). Chez les gros fumeurs, exposés professionnellement, le taux de bronchite atteint 63,5 % (près des 2/3 des sujets) 'Fig. 8).

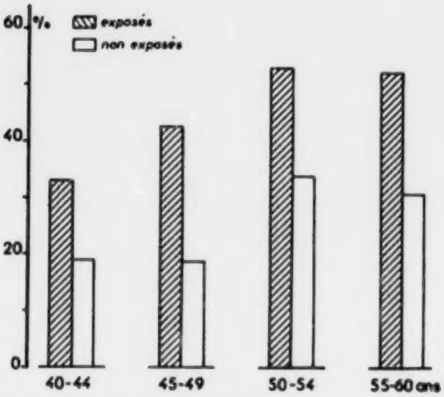

Fig. 7. Taux de bronchite selon age et expositions professionnelles.

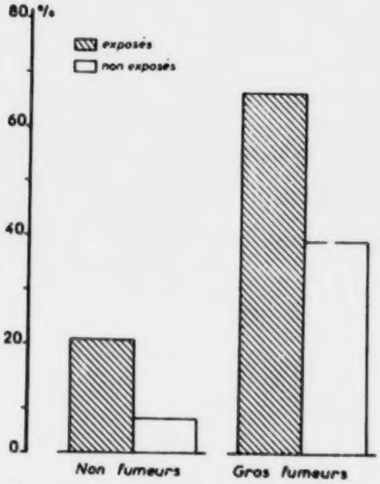

Fig. 8. Taux de bronchite suivant tabac et expositions professionnelles.

La fréquence de la dyspnée est aussi plus grande chez les gros fumeurs qui sont exposés professionnellement à d'autres polluants (Fig. 9). Quelques unes des données fonctionnelles, dont les valeurs moyennes sont rappelées dans le tableau II, montrent indiscutablement des perturbations plus marquées chez les sujets fumeurs et exposés professionnellement à des nuisances. Cette différence est surtout très significative pour TCO/V. La plus grande fréquence d'un syndrome obstructif (rapport VEMS/CV < 65%) est rappelée dans la fig. 10.

La nature de l'exposition professionnelle joue-t-elle un rôle ? Ce point a été particulièrement étudié dans le groupe des ouvriers sidérurgiques où les expositions sont les mieux caractérisées. Dans ce

CV %, VEMS % et TCO/m2 EN FONCTION DE L'EXPOSITION, DES HABITUDES TABAGIQUES ET DE L'AGE

	Non Exposé				Exposé professionnellement			
	40-44 ans	45-49 ans	50-55 ans	55-60 ans	40-44 ans	45-49 ans	50-55 ans	55-60 ans
				CV %				
Fumeur	106,17 (13,4)	95,16 (14,0)	96,23 (25,3)	91,95 (14,4)	100,40 (15,9)	98,68 (17,33)	96,09 (16,7)	93,98 (14,54)
Non Fumeur	105,10 (20,3)	102,73 (17,6)	94,83 (13,8)	101,00 (29,6)	104,23 (18,8)	95,40 (17,1)	98 (18,0)	92,9 (15,66)
				VEMS %				
Fumeur	105,00 (14,3)	96,14 (19,9)	95,32 (17,5)	89,2 (21,5)	100,65 (21,3)	98,23 (23,9)	95,87 (19,8)	88,57 (20,9)
	*				*			*
Non Fumeur	106,01 (28,3)	108,00 (19,5)	99,82 (15,2)	99,33 (27,1)	115,23 (24,3)	104,90 (27,7)	104,84 (18,1)	99,15 (20,3)
				TCO/m^2/min./mmHg (apnée)				
Fumeur	14,29 (6,1)	13,33 (3,1)	13,17 (2,52)	11,92 (2,7)	12,67* (3,1)	11,62** (4,0)	10,64*** (3,0)	9,94*** (2,6)
	*	***		***			**	**
Non Fumeur	18,25 (5,5)	19,36 (5,8)	14,20 (3,6)	17,60 (7,4)	14,29 (6,1)	13,33** (3,1)	13,17 (2,5)	11,92** (2,7)

Tableau II

Les astérisques placés après les chiffres des colonnes de droite comparent celles-ci avec les colonnes correspondantes, à gauche. * < 0,05 ** < 0,01 *** < 0,001.

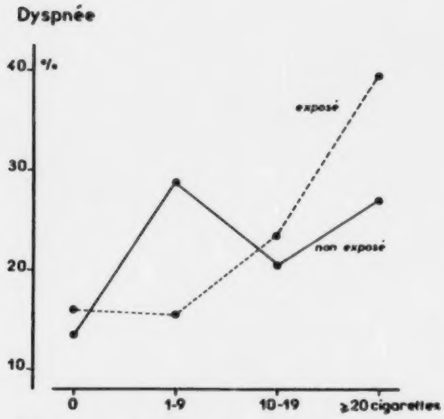

Fig. 9. Effets du tabac sur la dyspnée.

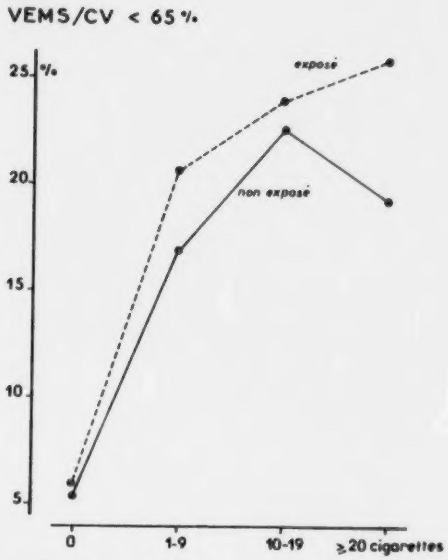

Fig. 10. Effets du tabac sur le VEMS/CV.

groupe, nous avons distingué les sujets sans exposition, ceux exposés uniquement aux poussières, et ceux ayant une exposition multiple, poussières fumées et vapeurs chimiques.

La fréquence de la toux et de l'expectoration chroniques est plus importantes chez les sujets exposés surtout aux poussières. Il n'y a pas de différence nette dans la fréquence de la dyspnée ou des poussées pulmonaires aiguës durant les trois dernières années. Les habitudes tabagiques sont comparables dans les trois sous-groupes selon la nature de l'exposition (Fig. 11). La comparaison des données fonctionnelles de ces trois sous-groupes est rappelée dans les tableaux III et IV. On ne constate pas de différence notable dans les valeurs spirographiques. Par contre, il existe une baisse significative de TCO/V et de la ductance du CO à l'effort dans les groupes soumis à une exposition multiple. Ces résultats font se demander si l'exposition aux polluants ne détériorerait pas plus spécifiquement l'efficacité des échanges, et soulignent l'intérêt pour les études épidémiologiques non seulement des techniques spirographiques classiquement utilisées, mais aussi des épreuves permettant de juger de l'efficacité des échanges, en particulier des épreuves au CO.

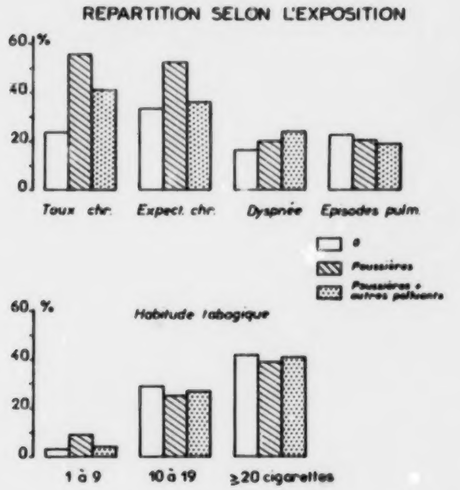

Fig. 11. Les différents effets pulmonaires et habitudes tabagiques en fonction de l'exposition.

DISCUSSION

Dans cette étude, nous nous sommes adressés à deux groupements socio-professionnels bien définis alors que le troisième groupe est constitué par les habitants de l'agglomération. Comme le souligne B. FERRIS (3) et FLETCHER (5), les groupes de travailleurs constituent des populations spéciales et sélectionnées. Les sujets doivent être en bonne santé pour pouvoir obtenir leur travail, d'autre part les personnes qui peuvent avoir un certain degré d'atteinte pulmonaire ne choisiraient pas le métier d'ouvrier métallurgiste mais plutôt un métier réputé peu exposé comme celui de commerçant par exemple. Ceci pourrait expliquer les différences que nous avons constatées du point de vue spirographique entre le groupe des ouvriers de l'usine sidérurgique avec les autres groupes. D'autant que pour le groupe des habitants de l'agglomération, nous n'avons pu faire

RESULTATS EN FONCTION DE L'EXPOSITION DURANT LE TRAVAIL

	I aucune exposition n = 31	II exposition aux poussières n = 64	III exposition aux poussières, fumées et vapeurs n = 98	Test t		
				I-II	I-III	II-III
CV % de la théorique						
M	98,60	99,20	99,90			
σ	19,30	16,70	16,80			
VEMS % de la théorique						
M	103,00	99,40	101,40			
σ	27,00	21,60	22,5			
VEMS/CV %						
M	76,50	74,40	74,70			
σ	9,20	13,00	10,70			
VR/CT %						
M	27,10	26,25	26,39			
σ	7,80	6,70	8,18			

Tableau III

(Ne figure pas dans ce tableau le sous groupe des 7 sujets exposés aux fumées et/ou aux vapeurs chimiques). Il n'y a pas de différence significative au point de vue spirographique selon les expositions professionnelles.

RESULTATS EN FONCTION DE L'EXPOSITION DURANT LE TRAVAIL

	I aucune exposition n = 31	II exposition aux poussières n = 64	III exposition aux poussières, fumées et vapeurs n = 98	Test t		
				I-II	I-III	II-III
REGIME STABLE						
TCO/m2, ml/min/mmHg						
Repos						
M	13,81	12,91	12,66			
σ	2,66	4,17	3,59			
Exercice						
M	14,77	13,69	13,35			
σ	3,92	3,60	3,96			
TCO/V						
Repos						
M	2,11	1,94	1,80			
σ	0,65	0,69	0,63		*	
Exercice						
M	2,62	2,63	2,34		*	*
σ	0,72	0,83	0,72			
APNEE						
TCO/m2						
M	13,55	12,58	12,60			
σ	2,50	2,72	2,73			
DuCO% de la théorique						
Repos						
M	99,10	99,70	98,30			
σ	13,40	10,60	12,00			
Exercice						
M	105,70	104,70	100,70			
σ	15,90	14,80	12,5		*	*

* = $p < 0,05$.

Tableau IV

Ne figure pas dans ce tableau, le sous-groupe composé de 7 sujets exposés aux fumées et/ou aux vapeurs chimiques. C'est surtout les mesures au CO à l'effort (TCO/V et ductance) qui distinguent le sous-groupe des sujets exposés aux polluants multiples.

les éliminations d'après les radiographies comme pour les deux autres groupes pour lesquels les radiographies systématiques de médecine du travail ont pu être faites tous les ans. Afin de rendre le plus comparable possible les groupes socio-professionnels, nous n'avons choisi que des sujets hommes, de 40 à 60 ans, en échantillons stratifiés par 5 ans d'âge et nous avons supposé qu'habitant la même petite agglomération les facteurs atmosphériques seraient les mêmes. En fait, selon les vents dominants, la répartition des fumées et poussières de l'usine sur l'agglomération peut être différente d'un jour à l'autre. Ceci étant, l'étude comparative qui a été faite ici permet de bien souligner, comme l'ont déjà fait LOWE et coll. (9), FRAPPIER-DAVIGNON et coll. (6), l'influence importante du facteur tabagique qu'il faut prendre en considération chaque fois que l'on veut étudier le rôle des pollutions dans les maladies pulmonaires chroniques. Loin cependant de conclure comme LOWE (9) qu'il s'agit du seul facteur important, les résultats ici présentés montrent que la pollution profesionnelle joue un rôle indiscutable. Les résultats montrent aussi qu'il y a un effet potentialisateur indiscutable du tabac et les facteurs de nuisance atmosphérique professionnelle. L'intérêt des épreuves au CO dans les enquêtes épidémiologiques est ici bien souligné par les différences observées seulement avec ces tests dans les groupes distingués selon leur type d'exposition professionnelle.

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DISCUSSION

LEFEVRE (Belgique)

Voulez-vous donner des précisions sur le calcul du facteur de transfert du CO envers les sujets fumeurs ou non fumeurs.

PHAM (France)

Ce facteur de transfert a été étudié par la technique en respiration unique (single breath) et par la technique en état stable. Pour chaque sujet, l'analyseur est reglé au point zéro sur l'air expiré du sujet (fumeur ou non fumeur) afin d'éviter les inconvénients de la "back pressure"

LÄRMBELASTUNG, KOHLENMONOXIDEBELASTUNG UND KOMBINATIONSWIRKUNGEN

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KURZFASSUNG

Für die Festlegung von Lärmgrenzwerten sind u. a. Untersuchungen der zeitweiligen Hörschwellenverschiebung (TTS) und des Hörerholungsvorgangs von Bedeutung. In unseren Experimenten konnte gezeigt werden, dass die Rückbildung der Hörermüdung bei gleichzeitiger Lärmbelastung schon ab 65 dB verzögert werden kann. Zwischen 75 und 85 dB steigt diese Verzögerungswirkung stark an und schlägt bei etwa 85 dB in zunehmende Hörermüdung um.

Über die Kohlenmonoxidwirkung auf den Menschen konnten wir eine Reihe psychophysiologischer Experimente durchführen, u. a. konnten dabei Auswirkungen auf die Informationsverarbeitung im Gehirn mit Hilfe von computeranalysierten Potentialen nachgewiesen werden. Um Aufschlüsse über mögliche Kombinationswirkungen zu erhalten, wurden 20 Versuchspersonen in einem Doppelblindversuch 4 Stunden mit 200 ppm Kohlenmonoxid belastet und während der letzten 1/4 Stunde einem Lärm von 105 dB ausgesetzt. Die Hörschwellenverschiebung und der Verlauf der Hörerholung (1 Stunde) zeigten keine statistisch signifikanten Unterschiede.

Während für hohe COHb-Werte synergistische Wirkungen mit Lärm auf das Hörsinnessystem beschrieben sind, zeigen diese Ergebnisse, dass bei geringen Dosen die beiden Umweltnoxen indifferent, also voneinander weitgehend unabhängig, wirken dürften. Der Lärm bewirkt die Hörschwellenverschiebung durch Einwirkung

auf die Haarzellen im Innenohr, während das Kohlenmonoxid eher die zentrale akustische Informationsverarbeitung beeinflusst.

(Mit finanzieller Unterstützung des österreichischen "Fonds zur Förderung der wissenschaftlichen Forschung" und der "Allgemeinen Unfallversicherungsanstalt".)

ABSTRACT

In determining limits for noise levels two of the important factors are investigations into temporary threshold shifts (TTS) and the hearing recovery process. In our experiments we were able to demonstrate that the regression of aural fatigue from coincident noise levels could be delayed from 65 dB upwards. This delaying effect increases sharply between 75 and 85 dB and at approximately 85 dB suddenly changes to an increase in aural fatigue.

We were able to conduct a series of psychophysiological experiments on the effect of carbon monoxide on man. One of our findings was evidence of the effects on data processing in the brain, which was obtained with the help of computer-analysed potentials. In order to obtain data on possible combined effects 20 subjects were subjected to 200 ppm carbon monoxide for 4 hours in a double blind test, and during the final quarter of an hour a noise at a level of 105 dB was added. No statistically significant differences were found between the temporary threshold shifts and hearing recovery time (1 hour).

While noise-related synergistic effects on the auditosensory system are described for high COHb values, these results show that the effects of both environmental contaminants, in small doses, are probably indifferent, and therefore mainly independent of each other. The noise, acting on the hair cells of the inner ear, produces temporary threshold shifts, while the effect of the carbon monoxide is rather on central acoustic data processing.

1) Einleitung

Lärm und Kohlenmonoxid sind zwei so weit verbreitete Umweltnoxen, daß es sehr wichtig erscheint, auch ihre möglichen Kombinationswirkungen sorgfältig abzugrenzen. Für hohe COHb-Werte konnte eine synergistische Wirkung - wahrscheinlich aufgrund der hervorgerufenen Hypoxidose im Innenohr - mit Lärm nachgewiesen werden. Für geringe Dosen müssen aber die möglichen Kombinationswirkungen noch eingehend untersucht werden. Einen Beitrag dazu sollen unsere Untersuchungen über Hörerholung unter Lärmbelastung sowie über Kohlenmonoxidwirkung auf das Zentralnervensystem und schließlich über das Zusammenwirken von Lärm- und Kohlenmonoxidbelastung bei der Beeinflussung von Hörermüdung und Hörerholung liefern.

2) Hörerholung unter Lärmbelastung

Der lärmbedingte Hörverlust beruht auf der Summierung wiederholter Gehörermüdungen. Die Hörschädigung wird durch eine Stoffwechselinsuffizienz der Innenohrhaarzellen bewirkt und schreitet umso intensiver fort, je häufiger ein noch ermüdetes Ohr neuerlichen Lärmbelastungen ausgesetzt wird. Untersuchungen über zeitweilige Hörschwellenverschiebungen (TTS) sind sehr wichtig, um die Gesetzmäßigkeiten der Hörermüdung und der Hörerholung aufzuklären und entsprechende prophylaktische Maßnahmen vorzuschlagen. In einigen experimentellen Untersuchungen (Schwetz et al. 1, Doppler et al. 2) konnten wir die Hörerholung bei gleichzeitiger Einwirkung eines weißen Rauschens bzw. eines Oktavbandgeräusches mit 2ooo Hz Mittenfrequenz untersuchen. Wir konnten dabei nachweisen, daß die gleichzeitige Geräuscheinwirkung die Gehörerholung stark zu reduzieren vermag. Bereits bei einem "Ruhelärmpegel" von 65 dB ist die Verzögerung der Hörerholung nachweisbar, wenn auch erst nach einer Stunde und nur in geringem Ausmaß. Bei einem "Ruhelärmpegel" von 75 dB oder zwischen 75 und 85 dB steigt die Verzögerungswirkung stark an und schlägt bei 85 dB und darüber in zunehmende Hörermüdung um. Die beobachteten Hörerholungskurven

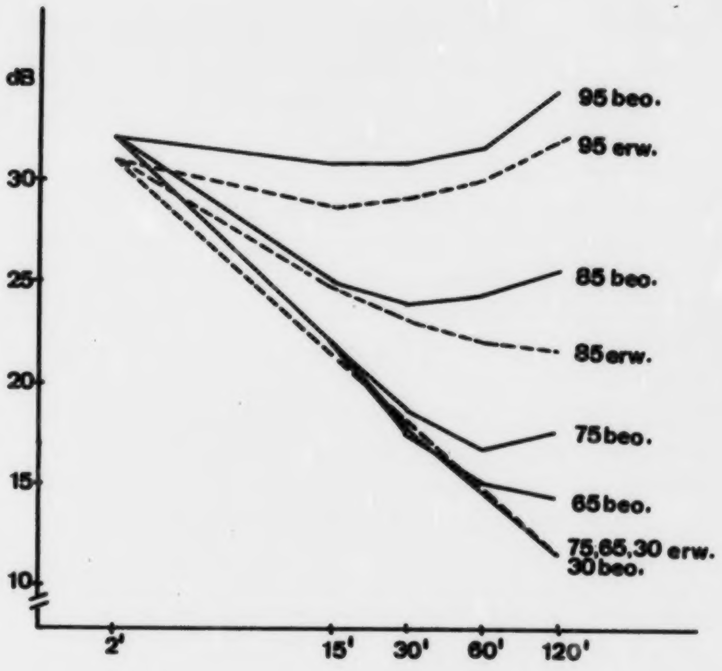

Abb.1 Zeitlicher Verlauf der Hörerholung bei gleichzeitiger Lärmeinwirkung von 3o, 65, 75, 85 und 95 dB (ausgezogene Kurven). Die strichlierten Kurven stellen die nach Formeln errechneten Werte dar. Nach einer Stunde weicht sogar die Hörerholung unter Geräuscheinwirkung von 65 dB von den berechneten Erwartungswerten ab.

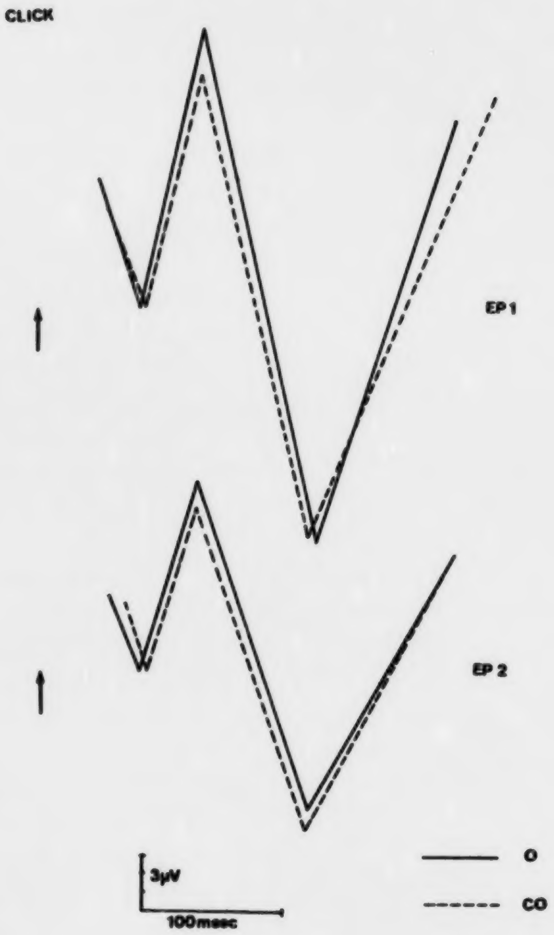

Abb.2 Akustisch evozierte Potentiale mit und ohne Kohlenmonoxideinwirkung. Die schematische Darstellung der späten Potentialanteile läßt erkennen, daß nur geringe Amplituden- und Latenzunterschiede bestehen.

sind in Abb.1 den nach Berechnungen (Ward 3) erwarteten Kurven gegenübergestellt.

3) Auswirkungen geringer Kohlenmonoxiddosen auf Hirnpotentiale und Hörfunktion

In einigen Versuchsreihen (Groll-Knapp et al. 4) konnten wir nachweisen, daß auch geringe Dosen von Kohlenmonoxid Veränderungen bei computeranalysierten Hirnpotentialen bewirken können. Die durch Clicks evozierten Potentiale zeigten dabei allerdings nur sehr geringe Unterschiede zwischen CO-Bedingung und Kontrollbedingung. Dies ist in Abb.2 dargestellt. Die Amplituden sind unter CO-Einwirkung etwas reduziert. Mehr Unterschiede zeigen die langsamen Hirnpotentiale. Solche bilden sich beispielsweise zwischen zwei Reizen, einem Warnreiz und einem imperativen Reiz, in Erwartungssituationen aus. Beispiele mit deutlichen Änderungen unter CO-Einwirkung gegenüber der Kontrollbedingung mit Normalluft sind in Abb.3 gezeigt. Offensichtlich kann die Informationsverarbeitung im Zentralnervensystem durch Einwirkung von Kohlenmonoxid auch in geringen Dosen gestört werden. Die Störung ist deutlicher bei elektrischen Hirnpotentialen, die mit komplexen Situationen, wie Erwartungsvorgängen, verknüpft sind.

Ototoxische Wirkungen des Kohlenmonoxids sind seit langem beschrieben, dürften aber erst bei höheren Konzentrationen auftreten. Hörverluste wurden vor allem nach Einwirkung von Generatorgas bei einem beträchtlichen Prozentsatz der untersuchten Personen gefunden. (Lumio 5)

4) Kombinationswirkungen von Lärm und Kohlenmonoxid

Es gibt eine Reihe von klinischen Beobachtungen über Arbeitssituationen, bei denen sowohl Lärmbelastung als auch Kohlenmonoxidbelastung vorlagen. Es ist dabei schwierig festzustellen, ob die eine oder die andere Noxe sich in einer Hörstörung auswirkte, oder ob beide Noxen eine kombinierte Wirkung hatten (Wagemann 6). In Tierversuchen wurde einerseits eine durch Lärm verzögerte Kohlenmonoxidausscheidung

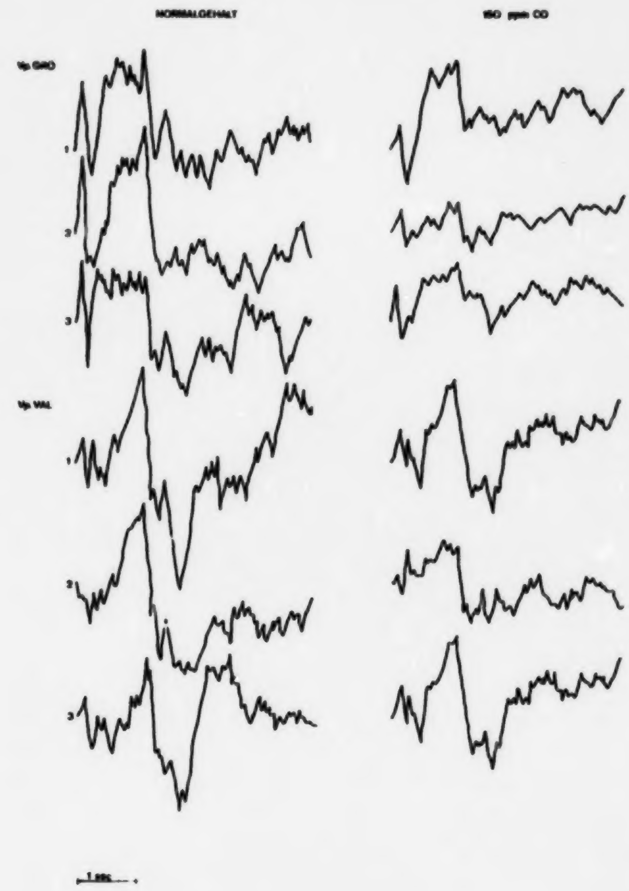

Abb.3 Beispiele von Erwartungswellen mit und ohne Kohlenmonoxideinwirkung. Die untereinander gezeichneten 3 Kurven jeder Vp. entsprechen den 3 Abschnitten eines Vigilanztests.

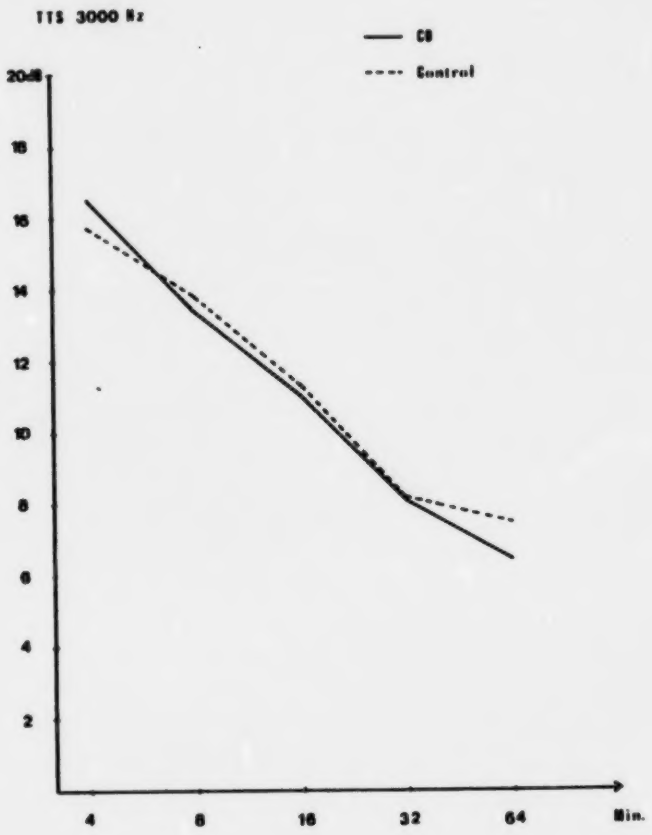

Abb.4 Zeitlicher Verlauf der Hörerholungskurven nach 15 Minuten Lärmbelastung (1o5 dB Oktavbandlärm, 2ooo Hz Mittenfrequenz). Die ausgezogene Kurve mit vorheriger CO-Belastung und die strichlierte Kurve ohne CO-Belastung zeigen nur geringe, statistisch nicht signifikante Unterschiede.

behauptet (Zorn 7) und andererseits treten durch Kohlenmonoxid bewirkte histologische Veränderungen an den Haarzellen bei gleichzeitiger Lärmeinwirkung früher ein (Kittel und Theissing 8). In diesen Fällen ist also eine synergistische Kombinationswirkung gegeben. Die COHb-Werte lagen allerdings in den letztgenannten Untersuchungen sehr hoch. Sie betrugen 5o% und mehr.

Um Aufschlüsse über mögliche Kombinationswirkungen auch bei Einwirkung geringerer Kohlenmonoxiddosen zu erhalten, haben wir die Hörermüdung (TTS) und die Hörerholung mit und ohne vorherige CO-Einwirkung bei 2o Vpn untersucht. Sie wurden in einem Doppelblindversuch entweder einer Atmosphäre von 2oo ppm CO oder normaler Atemluft für je 4 Stunden ausgesetzt. Während der letzten 15 Minuten wurden sie jedesmal mit einem Oktavbandlärm von 1o5 dB und einer Mittenfrequenz von 2ooo Hz beschallt. Die zeitweilige Hörschwellenverschiebung wurde mit Testtönen von 3ooo und 4ooo Hz nach 4, 8, 16, 32 und 64 Minuten geprüft. Die Hörerholungskurven für Testtöne von 3ooo Hz sind in Abb.4 dargestellt. Die TTS-Werte nach kombinierter CO- und Lärmeinwirkung sind bei der ersten Messung etwas höher als in der Kontrollsituation, bei den weiteren Messungen aber praktisch gleich und bei der letzten Messung nach einer Stunde sogar etwas niedriger. Keiner der Unterschiede war statistisch signifikant. Die COHb-Werte liegen zum Zeitpunkt der Beschallung bei ca. 13%. Bis zu dieser Höhe verhalten sich demnach Kohlenmonoxidbelastung und Lärmbelastung hinsichtlich der zeitweiligen Hörschwellenverschiebung indifferent. Die Ursache liegt wahrscheinlich darin, daß Kohlenmonoxidwirkungen in geringen Dosen mehr zentral angreifen, also die akustische Informationsverarbeitung im Rahmen komplexerer Aufmerksamkeits- und Erwartungsvorgänge beeinträchtigen, während Lärm hinsichtlich der TTS vorwiegend die Funktion der Haarzellen im Innenohr herabsetzt. Synergistische Wirkung zwischen Lärm und Kohlenmonoxid wird offensichtlich erst dann feststellbar, wenn auch die Hypoxidose in den Haarzellen des Innenohres durch Kohlenmonoxid allein ein merkbares Ausmaß erreicht.

Es wird Aufgabe weiterer Untersuchungen sein festzustellen, ob, wann und unter welchen Umweltbedingungen mit dem Beginn synergistischer Wirkungen zwischen Kohlenmonoxid und Lärm beim Menschen zu rechnen ist.

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DISKUSSION

JANSEN (Bundesrepublik Deutschland)

Ihre Beobachtungen über Hörermüdungen stimmen nicht mit denen Von Ward überein. Dies ist auch anderen Forschern aufgefallen. Ward erklärte, dass seine Beobachtungsformeln nur für einmalige Schallereignisse gelten. Frage: Haben Sie nur "einmalige" oder "intermittierende" Geräusche in Ihren Untersuchungen angewandt?

HAIDER (Oesterreich)

Wir haben zwar zur Erzeugung der Hörermüdung dieselben Versuchsbedingungen wie Ward angewandt, haben aber die Hörerholung länger beobachtet als Wart dies ursprünglich tat. Daraus ergaben sich die Unterschiede. Wart hat in neueren Untersuchungen unsere Ergebnisse bestätigt.

REVALUATION OF EPIDEMIOLOGICAL HEALTH EFFECTS FORMERLY ATTRIBUTED TO MEASURED LEVELS OF NITROGEN DIOXIDE IN VIEW OF SYNERGISTIC EFFECTS DUE TO CO-POLLUTANTS

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Detroit, Michigan, USA

ABSTRACT

Data have been collected to indicate the presence of several synergistically interfering air contaminants in ambient air in the immediate vicinity of Chattanooga, Tennessee, where epidemiological studies have classically related observed human respiratory effects to measured elevated levels of NO_2 *alone.*

Such data appear to support recent evidence showing the relationship between levels of small particle sulfate aerosol and indicence of respiratory disease.

The possible synergism between sulfate aerosol and NO_2 *is discussed and compared to data from the recent U.S. Government Community Health Environmental Systems Study (CHESS) particulate in the particle size range below 3.5 microns.*

Levels of aerosol sulfate recently measured in an urban industrial area are presented together with sulfate distribution as a function of particle size range.

It follows from these data that adverse health effects classically attributed to nitrogen dioxide may be accounted for either totally or in part by the presence of epidemiologically interfering compounds such as aerosol sulfate or acid mist whose specific measurements have not previously been a part of epidemiological surveys.

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Introduction

Between November 1968 and April 1969, a study of "acute respiratory illness" was conducted among families residing in the areas of the Greater Chattanooga School District which includes Hamilton County, Tenn., and Walker County, Ga. These areas are in close proximity to a large TNT plant which may be considered responsible for elevated levels of nitrogen dioxide for which reason the area was chosen as a single-pollutant related population study.[1]

Conclusions flowing from this study have been employed as classical evidence of human health effects attributable to exposure to ambient levels of nitrogen dioxide. Evidence contained in this discussion would, however, suggest that in addition to NO_2 emissions there were, in fact, significant quantities of other TNT process-related contaminants (acid mists such as nitric and sulfuric) on a scale which would preclude the assumption that the study area represents an environment in which observed respiratory symptoms may be related exclusively to nitrogen dioxide exposure.

Consideration 1: Discussion of the Physiological Effects of Human Exposure to Nitrogen Dioxide as These Relate to the Chattanooga Study

Since ventilatory performance has been related to NO_2 exposure by reason of a number of studies[2] and because toxic properties of NO_2 are usually attributable to irritation of mucous membranes, it was apparently determined that ventilatory performance would be used in the Chattanooga Study as an index of NO_2 exposure in school children together with information gained through questionnaires.

While a number of socioeconomic factors in addition to home exposure to cigarette smoke was considered and resolved by Shy *et al.*, [1] exposure to other TNT production related copollutants which included the synergistically related particulate-SO_2(SO_3) couple was apparently considered negligible or equivalent in comparing test areas.

Consideration 2: Discussion of the Physiological Effects of Human Exposure to Sulfuric Acid Mist, Nitric Acid Mist, and Sulfur Dioxide as Physiologically Interfering to the Effects of Nitrogen Dioxide

As described in Air Quality Criteria for Sulfur Oxides, physiological response to sulfur dioxide and sulfur trioxide are as follows:[3]
- Incidence of chronic pulmonary disease
- Prevalence of respiratory symptoms
- Changes in ventilatory function

Here it appears from reference to Consideration 1, that ventilatory functions are affected in the same manner by sulfur oxides as by nitrogen dioxide. In this regard, reference to the difficulty in obtaining even interference-free sulfur oxide atmospheres for the purpose of studying specifically SO_x community epidemiology is presented in Air Quality Criteria for Sulfur Oxides.[3]

Such combined exposures which are also typified by "increase in pulmonary flow resistance,"[3] would be expected to produce a sensitized individual on the basis of laboratory experiments which show, "A three to four fold potentiation of the irritant response . . ." [3] when conditions of synergistic exposure are observed.

Such sensitization has appeared as a result of synergistic exposure tests involving sulfur dioxide where successive exposure to contaminated air has produced a post exposure period of 1-5 hours during which pulmonary resistance continued at test level or even increased slightly.[3]

As a further example of post exposure sensitization such contaminants as carbon monoxide which are retained by the body in equilibrium for several hours after exposure are known to produce human systemic imbalance as measured by elevated blood carboxyhemoglobin, where several researchers have related this imbalance to sensitization or potentiation to develop high blood chlolesterol levels[4] of the degree associated with arteriosclerosis. Indeed, it is the post exposure sensitivity of siblings and their families to contract respiratory illness which is the basis upon which Part II of the classical NO_2 population exposure study in Chattanooga was founded.[5]

Consideration 3: Major Non-Nitrogen Oxide Emissions in the Control Area

In evaluating the presence and degree of symptoms attributed purely to NO_2 in this classical study, it is important to note that according to a *Summary Report of Air Pollution Evaluations* prepared by the U. S. Army Environmental Hygiene Agency, Edgewood Arsenal, the boiler plant proximate to the TNT plant at Tyner, Tenn., which is adjacent to the test area, produces daily the following non-nitrogen oxide emissions:

25,500 lbs of particulates - daily [6]
16,100 lbs of sulfur oxides - daily [6]

while the Sellite stack emits:

23,900 lbs of sulfur dioxide - daily [6]
2,120 lbs of 100% sulfuric acid mist - daily [6]

Equally important, as noted among the conclusions of this emissions inventory,[6] is the statement, "Oxides of nitrogen sulfuric and [*sic*] nitric acid mist, sulfur dioxide, and particulates are all being presently emitted in quantities sufficiently large as to have air pollution potential."

In brief, while a maximum of only 96,400 lbs/day of NO_2 emission is accounted for, 1.5 times this quantity or 151,100 lbs/day of *other respiration relatable contaminants* are co-emitted.

Evidence of these concentrations of equally or more harmful air contaminants exists in the community as measurable levels of sulfur dioxide in the range of 0.032 to 0.047 ppm accompanied by sulfuric acid mist at levels of 1.1 to as much as 13.0 mg/m^3.[6] These levels may be the result of recorded Oleum-Sellite plant source emission volumes of 9,300 ppm sulfur dioxide together with 326,000 μg/m^3 of total acid mist.[6]

Supplementary to these measurements are percentage sulfates found in suspended particulates, reported during the 1969 Study by Shy, *et al.*[1] These findings show high levels of suspended sulfate in the NO_2 area which vary from an overall mean of 11.2 μg/m^3 particulate sulfate to a 90th percentile average high of 20.7 μg/m^3. Since these relatively high suspended sulfates are measured in a low sulfur dioxide air shed, it is certainly a valid inference that otherwise unaccounted for sulfuric acid mist is responsible for the balance of sulfate and at least a portion of respiratory symptoms classically attributed purely to nitrogen dioxide. The inference attached prima facie, to the presence of sulfate aerosol is disquieting in the light of recent evidence observed in the reports of the U. S. EPA Community Health Environmental Systems Study (CHESS). Published data resulting from this study of five major U. S. areas indicate significant correlation between incidence of respiratory health effects and measured elevated levels of sulfate aerosol.[7] To date, no certain lower limit of sulfate aerosol has been attributed to observed health effects. Detroit, Michigan measurements show sulfate concentrations averaging 11 percent of suspended dust in the respirable range below 10 µ diameter over the five year period ending in 1970.

Assessment of epidemiological symptoms attributed to nitrogen dioxide may be accounted for by one or more of the following alternatives.

Possible Explanations of Observed Respiratory Symptoms Attributed to NO_2

a. Symptoms of air-related respiratory disorders are the product of a synergistic effect resulting from exposure to a combination of pollutants, i.e., nitrogen dioxide and combined sulfuric acid and nitric acid mist.

b. Symptoms which result from exposure to levels of nitrogen dioxide greater than 0.083 ppm are observed in a population which is rendered sensitive by virtue of background exposure to steady and/or intermittent high levels of combined acid mist (HNO_3 and/or H_2SO_4).

c. Symptoms are a result of exposure to acid mist only, rather than to actual nitrogen dioxide levels measured.

Conclusion

We conclude that the basis for epidemiological health effects representing long term exposure of populations to NO_2 at ambient levels of 0.05 ppm or less is faulty and founded upon inappropriate evidence.

As a result, we conclude that the degree of hazard attached to long term, low level exposure to NO_2 is unnecessarily strict and represents rather the sensitivity of individuals who are coincidentally exposed to substantial levels of other non-nitrogen dioxide pollutants (H_2SO_4 and HNO_3 mists) which are equally or more hazardous than NO_2!

We encourage further research to better elucidate population health effects attributable to nitrogen dioxide; meanwhile, we would identify as premature any extraordinary measures to drastically reduce or eliminate exclusively atmospheric emissions of nitrogen dioxide, in view of evidence which suggests the equal or greater need to limit respiratory burden of coambient aerosol particulate sulfate and acid mist.

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DISCUSSION

STEENSBERG (Denmark)

The paper is of particular interest because the Chattanooga studies have been an important basis for considering criteria for nitrogen oxides. Even on this basis - which now seems to be too weakly founded - the working party behind the WHO technical report no. 506 did not find sufficient basis for establishing NO_x criteria. I am therefore interested to know whether other studies are under way on the influence of nitrogen oxides on human health.

WARNER (U.S.A.)

It is my understanding that the Chattanooga area is being restudied. In addition, another study, EPA's Community Health Environmental Systems Study (CHESS) is presently in progress, and it is hoped that this study will better elucidate the relationship between measured levels of air contaminants and observed health effects.

Here, the investigation of the health effects attributable to sulfate aerosol forms an especially interesting aspect of the CHESS study.

VELI-PEKKA LEHTO (Finland)

Is it reasonable to conclude that the NO_2-limit is too strict, if it would lead to raising the permited level of NO_2. Is it not better to say that the NO_2-limit seems to be safe if we want to develop safe limits.

WARNER (U.S.A.)

This is a question of degree. What degree of control of NO_2 must be exercised in order to achieve the degree of safety required for reason of human health? Perhaps future studies will show that our efforts could be better spent controlling air pollutants exclusive of NO_2 that are more obviously health related.

BATES (Canada)

Was the source of SO_2 in Chattanooga sufficiently close to the source of NO_x, that the contours of the NO_x levels in Chattanooga would also be the contours of maximal distribution of sulphate?

WARNER (U.S.A.)

The sources of SO_2, sulphate (as acid) and NO_x were from the same plant. Damage to vegetation characterized as "acid damage" was reported in the U.S. Army industrial Hygiene Study. This particular acid-sulphate damage was reported within a 1 mile radius of the plant. High and moderately high suspended sulphates and nitrates were also observed; these over most of the study and control areas of Greater Chattanooga.

In other words measured levels of sulphate and nitrate as well as acid damage to vegetation are reported in the study area.

FREEMAN (U.S.A.)

The data presented refer to stable sources of NO_2 emissions only in view of the NO_2 dependent levels of ozone on photochemically generated pollutants, can the interdependent NO_2 level alone be considered by itself circumspectly?' Ozone, dependent upon NO_2 , is 10-20 times as toxic as NO_2 itself. What is the thinking in this regard?

WARNER (U.S.A.)

This is an interesting question. Stationary or stable NO_2 sources, rather than traffic sources of NO_2, would hopefully lead to a better, more ozone free examination of NO_2 effects. It is not altogether clear from the study data whether or not traffic related hydrocarbons were eliminated as as possible ozone producing interference.

ON THE INTERACTION OF ATMOSPHERIC POLLUTANTS

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ABSTRACT

In evaluating the health effects of chemical and physical factors, it is of great importance to know the possible interactions between different pollutants. The biological effects of interactions, when present, may be synergistic, antagonistic or additive. Each type of interaction calls for a different evaluation and different practical measures. As yet the understanding of such effects is not clear, probably because of differing definitions of terminology. For example, the combined effect of sulfur dioxide and particulates is interpreted as a synergistic effect; in the author's opinion, this is an aggravating effect. The type of interaction depends on the levels of concentration observed - for example, the synergism shown at high levels of concentration is not always demonstrated for low levels of concentration. In fact there is little evidence of synergistic effects from ambient air pollutants; the more common type of interaction is ***additive*** *in effect.*

Examples of each type of interaction will be presented.

Recent years have witnessed a noticeable growth in the interest towards the interaction of different atmospheric pollutants and industrial toxins. More and more facts are being amassed proving that the combined presence of two and more substance in the air that is inhaled can significantly change the degree of the toxic effect that is characteristic of each component of the mixture. Moreover, the ultimate effect of the combinedaction may be characterized as summation (total or partial), potentiation or antagonism.

It is quite understandable that the nature of the combined action is of considerable practical significance, first and foremost, when establishing air quality standards, both for atmospheric air and air in industrial premises.

The character of the combined action (summation, potentiation, antagonism) depends not only on the direction of the effect of each component in the mixture, but also on the level of active concentrations, for instance, lethal concentration levels and chronic action threshold, or even maximum permissible concentrations (MPC).

An experimental investigation aimed at verifying this supposition has revealed that narcotic substances of one group, in case of combined action, usually sum up, bothat lethal concentration levels and at chronic action threshold level. As for substances from different groups, for instance, narcotics and irritants, then the transition from lethal level to threshold level proceeds according to various laws, depending on concrete toxins: either a summation is observed, or an "independent" action according to Elkins classification. Independent action, according to our data, may sometimes be classified as sub-clinical antagonism.

Experience points to an exclusively rare appearance of potentiation in case of chronic combined action at chronic action threshold level. As an example, we may refer to the clinical observations when there was a combined action of ethanol and arsenic, ethanol and thiuramdisulfide, ozone and sulphuric acid aerosol by A.I. Nevskaya and T.A. Kochetkova [1].

The significance of the active levels may be demonstrated using the example of the interaction of SO_2 and NO_2. For the range of concentration in the open atmosphere, this is summation of effects (O.I. Shalamberidze [2]), and for a higher level it is antagonism (L.S. Mitina [3]).

The experience gained by Soviet hygienists in their study of the combined action of different chemical substances within the range of concentrations typical of the outside atmosphere (25 combinations), has led to the conclusion that the most frequent character of this action is total summation of effects (K.A. Buŝtueva [4]). Only for certain mixtures was there independent action (SO_2 + CO).

The nature of the combined action of several atmospheric pollutants has been taken into consideration in sanitary legislation for practical purposes.

The most complicated question is that of evaluating the character of the combined action of gases and aerosols. One of the prime difficulties in making such an evaluation is that besides the changes in the organism's reaction, the presence of aerosol can alter the depth to which the gas penetrates, and only on account of this, the character of the effect may change. And the latter can no longer be considered the result of the genuine interaction of the components.

In particular, an example of the latter can be seen in the combination of the inertly suspended sulfur dioxide cinders in the period of toxic fogs in London.

It is known that sulfur dioxide is a compound that dissolves well in water, as a result of which it is absorbed in the main in the upper respiratory tracts. Even in case of high concentrations, the absorption of SO_2 in the upper respiratory tracts varies from 48% to 81.3%, giving an average of 61.6% (I.D. Gadaskina [5]). In the presence of aerosols and high humidity, SO_2 dissolves in the moisture that condenses around the aerosol particles. On the aerosol particles the SO_2 is "conducted" to the alveolae where it is released in view of the great affinity in the lung tissue, creating a high local concentration. This, as could have been expected, lies at the basis of the unfavorable consequences of toxic fogs.

The first experimental proof that an aerosol could alter an organism's response to an irritant gas was presented by Dautrebande in 1939. He termed such an aerosol a "vector", and concluded that the adsorption of a gas on aerosol particles boosts the number of gas molecules that reach the lung alveolae.

This process was studied more intensively in the 1950s. The significance of size of particles was noted (Dautrebande L.,et al. [6]), as well as the physico-chemical peculiarities of gases and aerosols (La Belle C., et al.), the level of active components (Amdur M., [7]), the affinity of gases to aerosol particles in lung tissue (Goetz A., [8]).

Therefore, in the period of toxic fogs there was an intensification of the effect because of the change in the site where SO_2 came into contact, and not due to interaction in the scientific understanding of this process. Nevertheless, it is necessary to emphasize that for practical purposes, the very fact that the effect is intensified is what is important, not its scientific interpretation.

Another explanation of the types of combined action of gases and aerosols should be sought in the reactions of the organism. For example, in case of intratracheal introduction of copper oxide the fibrosis process in the lungs weakened if this was followed by prolonged inhalation of low concentrations of nitric oxides by the animals (I.V. Sanotsky, I.P. Ulanova, N.M. Karamzina, T.A. Kochetkova [9]).

The presence of copper oxide in the lungs weakened the irritating action of nitric oxides. In other cases, for instance, under the action of coal dust and carbon monoxide (V.K. Navrotsky [10]), or silicon oxide and radon (V.S. Kushneva [11]) pulmonary fibrosis intensified.

The simple summation effect was illustrated upon the interaction of sulfur dioxide and sulfuric acid aerosol in low concentrations (K.A. Buŝtueva [12]).

In industrial hygiene, when dealing with relatively constant mixtures of substances whose composition is not fully known, the standards are figured out according to the leading component which determines the clinical character of the mixture's action as a whole. Simultaneously it is necessary to take into consideration the need for hygienic standardization according to the most typical component which characterizes the source from which the toxins are emitted.

A number of formulae and charts have been proposed for evaluating the nature of the combine action of different chemical substances. However, the main shortcoming of the majority of these formulae for interaction of substances, in our opinion, is that they are based on the false supposition that at different quantitative levels the direction of the combined action of the toxins is the same. However, their practical usage is very limited without taking a differentiated approach, with due consideration to the latter factor.

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EXPOSITIONSINDIKATOREN
INDICATORS OF EXPOSURE
INDICATEURS D'EXPOSITION
INDICATORI DI ESPOSIZIONE
INDICATOREN VAN EXPOSIE

Vorsitzender - Chairman - Président - Presidente - Voorzitter

J.L. MONKMAN (Canada)

DISCUSSION

JOOSTING (Nederland)

Please would you be so kind as to tell us something more about the ways inhaled aerosols can influence the effect of other inhaled substances, e.g. gases. It seems to me that the uptake of gases by the lung and their effect would not be influenced by e.g. the harrowing effect of an inhaled aerosol on the lumen of the airways, because breathing will continue. Please would you correct me if I didn't understand you well?

BUSTUEVA (U.S.S.R.)

The interaction of inhaled aerosols and gases has an appreciable influence on their biological effectiveness.

The overall effect depends not only on the depth of penetration into the lungs, but also on the speed of adsorption and, most important, of desorption of the toxin from the surface of the particles (Sanockij, I.V. (1969) Toksikologija novyh promyšlennyh vešěstv (Toxicology of new industrial substances) v.II, p. 6).

The combined effect usually diminishes when there is significant interaction between gas and aerosol (hydrocarbons + A_2O_3).

Litau, V.G. & Solov'ev, V.I. (journal Gigiena turda i Profzabolevanija, 1973, No. 9, P. 58) detected a sharp increase in the action of steam products of thermal destruction of oils (ethers, aldehydes, ketones, organic acids and carbon monoxide) in the presense of oil vapour, which cannot easily be explained as being due to deep penetration of this mixture into the lungs, or to the processes of adsorption and desorption of the toxin from the surface of the particles. The indirect effect of oil vapour on the phospholipid marginal layer of the respiratory se of the lungs (sulfactant) is of significance.

MERCURY AND OTHER ELEMENTS IN BLOOD OF THE DUTCH POPULATION

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ABSTRACT

For a number of years problems have existed about the toxicity of heavy metals and other elements occurring in the environment in higher levels than in the past. Concern about this started with the Minamata disease in Japan, caused by methylmercury in fish. Also reports from Sweden about fish caught in lakes polluted with mercury caused some alarm. To see whether the Dutch population, especially people eating much fish, were in danger of (methyl)mercury intoxication, the mercury content of blood and hair was determined in a number of people. In this population the relation between fish-eating habits and mercury content was studied. People were asked whether they ate fish at least once a week, at least once a month, at least once a year or never and were divided into 4 groups accordingly. Questions about fish eating were asked for sea-fish and for freshwater fish, because the mercury content of the latter is generally higher. From the results a clear correlation was found between fish eating habits and mercury content of the blood. This correlation was found especially when freshwater fish was considered. However, the mercury content of the blood was, even for the heaviest fish eaters, relatively low compared with the results for Swedish and Finnish fish eaters and much lower than the level at which clinical symptoms can be expected.

The mercury content of hair did not correlate very well with blood, although in cases of relatively high mercury content of blood, the level in hair was also higher. But in general, for screening purposes of a population, mercury content of hair is not a valuable parameter. From this study the conclusion can be drawn that as yet no necessity exists for limiting the fish consumption of the Dutch population.

Introduction

In order to evaluate the possible risk of (methyl)mercury intoxication resulting from fish consumption, blood and/or hair was collected from people with known fish eating habits for determination of the total mercury content. Because people with a high and a low fish consumption were taken, it could be studied whether fish eating caused an increase in body burden of mercury. In the meantime we wanted to get an impression about the mercury content in blood of the Dutch population, compared with people from other countries. Hair was included in this study to examine whether mercury in hair could be used as a reliable index for determination of the total body burden of mercury.

2. Materials and methods

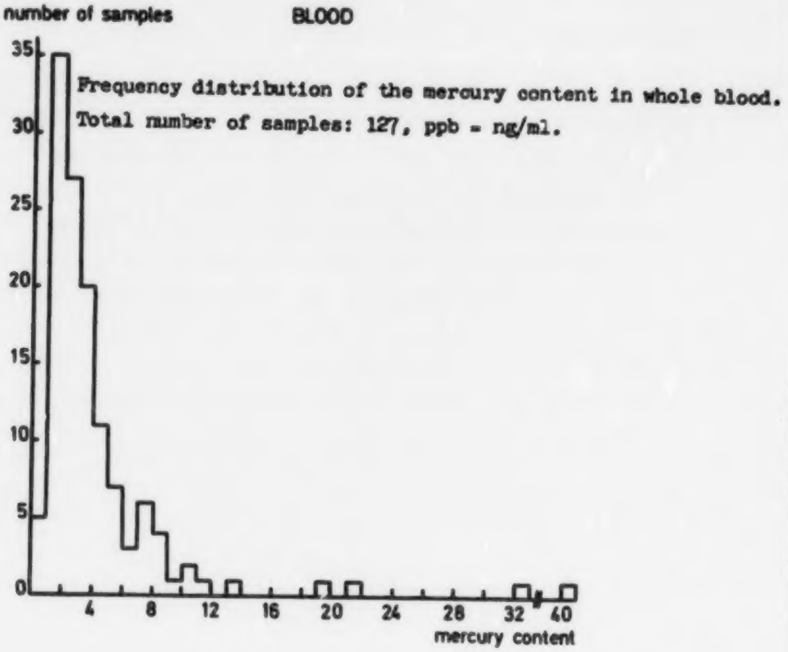
Blood was obtained from 127 and hair from 87 persons. People were questioned about their fish eating habits, graded in eating sea-fish at least once a week, at least once a month, at least once a year or never. The same was asked for the consumption of freshwater fish, since this is known to have in general a higher mercury content than sea-fish. A special effort was made to get persons who told to ate much or on the contrary never fish. The people involved were partly from our own institute or found with the help of some hospitals. In that way we also got samples from a little fishing-town called Volendam, where people are known to catch and eat much eel (freshwater fish).

Blood was sampled in heparinized tubes and the mercury content was determined in whole blood by means of neutron activation analysis. Mercury in some samples of hair was also determined by neutron activation analysis, but in most samples by atomic absorption spectrometry. These samples were washed thoroughly before analysis.

3. Results

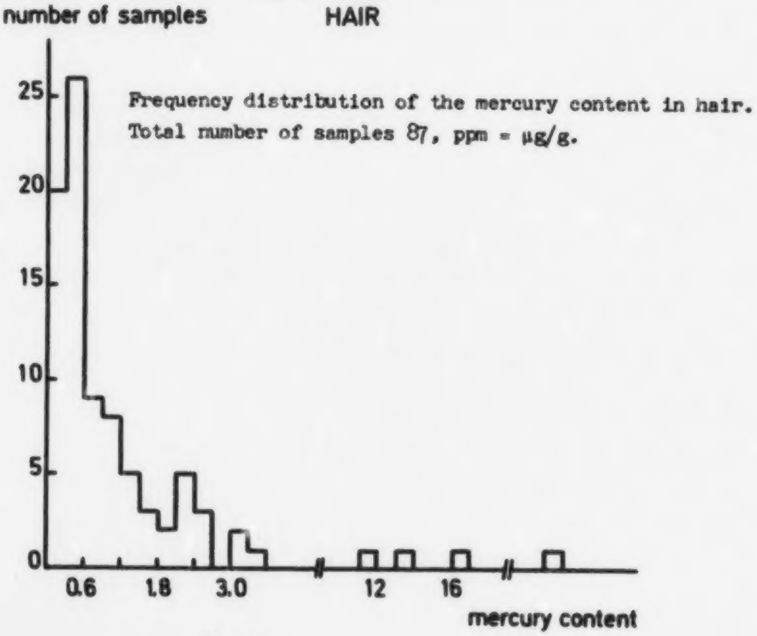
Blood

From the frequency distribution (fig.1) it can be seen that mercury in blood does not show a normal distribution. Therefore the



Frequency distribution of the mercury content in whole blood. Total number of samples: 127, ppb = ng/ml.

Fig I.



Frequency distribution of the mercury content in hair. Total number of samples 87, ppm = μg/g.

Fig 2.

Table 1. Mercury content in whole blood compared with consumption of sea-fish or freshwater fish

	relative fish consumption x)			
	3	2	1	0
sea-fish				
number of samples	49	46	18	13
median value (ppb)	4.4	2.4	2.0	1.5
range (ppb)	1.3-40.5	1.0-10.7	0.8-9.9	0.3-4.0
% > 4.0 ppb	53	24	11	0
freshwater fish				
number of samples	20	32	40	34
median value (ppb)	7.3	3.2	2.4	1.9
range (ppb)	1.8-40.5	1.3-32.5	1.0-10.7	0.3-7.3

x) 3 = at least once a week, 2 = at least once a month, 1 = at least once a year and 0 = never

Tabel 2. Statistical evaluation of the results from table 1 after logarithmization of the figures

	relative fish consumption			
	3	2	1	0
sea-fish				
number of samples	49	46	18	13
log 10x ± SD	1.69±0.33	1.43±0.23	1.32±0.28	1.16±0.26
P gr 3 / gr 2, 1 and 0	-	< 0.001	< 0.001	< 0.001
P gr 2 / gr 1 and 0	-	-	N.S.	< 0.001
freshwater fish				
number of samples	20	32	40	34
log 10x ± SD	1.86±0.32	1.54±0.28	1.41±0.24	1.31±0.29
P gr 3 / gr 2, 1 and 0	-	< 0.001	< 0.001	< 0.001
P gr 2 / gr 1 and 0	-	-	< 0.01	< 0.01

median value instead of the mean was estimated. This was 2.9 ppb (ng/ml), range 0.3-40.5 ppb.

From the frequency distribution it was found that

4% is 0 - 1 ppb
32% is 0 - 2 ppb
68% is 0 - 4 ppb
83% is 0 - 6 ppb
90% is 0 - 8 ppb
94% is 0 - 10 ppb

Only 8 values were higher than 10 ppb, varying from 10.7-40.5 ppb. To correlate the values in blood with fish consumption the samples were divided into 4 groups, of which group 3 consisted of people who according to their own statement ate fish at least once a week. Samples of people eating fish at least once a month, once a year or never were divided into group 2, 1 and 0 respectively. This was done for sea-fish as well as for freshwater fish. The results of the mercury content in blood for each group are summarized in table 1. Because the values do not meet the requirements of a normal distribution statistical analysis was carried out with the logarithms of the figures. After multiplying the figures by a factor 10, Student's -t-test was carried out on the logarithms. The results are given in table 2.

From the tables it is obvious that a dose response relationship is found between mercury in blood and fish consumption. There is a marked difference in median and log value, especially between group 3 and the other groups. According to statistical analysis the mercury content in blood of people in group 3 is significantly higher than that of the other groups, for sea-fish as well as for freshwater fish. In addition the mercury content in blood of people in group 2 is significantly higher than that of group 0 for sea-fish and of group 1 and 0 for freshwater fish. In all cases the differences are more marked when freshwater fish is taken into consideration. This was expected since it is generally known that the mercury content of this type of fish, like eel, pike and pike-perch is higher than that of sea-fish.

Table 3. Mercury content in whole blood of people eating much sea-fish, but less freshwater fish, compared with people eating little or no fish at all

	relative sea-fish consumption	
	3 x)	1 + 0
number of samples	30	31
median value (ppb)	3.6	1.7
range (ppb)	1 - 32.5	0.3 - 9.9
log 10x ± SD	1.57±0.28	1.25±0.27
P gr 3 / gr 1 and 0	-	< 0.001

x) Only people eating sea-fish at least once a week, but less freshwater fish

Table 4. Mercury content in hair compared with fish consumption (sea- and freshwater fish combined)

	relative fish consumption			
	3	2	1	0
number of samples	17	35	21	9
median value (ppm)	1.6	0.5	0.5	0.3
range (ppm)	0.6-13.2	0.2-3.5	0.2-3.2	0.1-2.6
% > 0.9 ppm	94%	17%	19%	22%
log 10x ± SD	1.26±0.29	0.71±0.28	0.76±0.34	0.64±0.44
P gr 3 / gr 2, 1 and 0	-	< 0.001	< 0.001	< 0.001

Because a number of people with a high consumption of sea-fish were also eating much freshwater fish, it is difficult to estimate the share that sea-fish has in the ultimate mercury content of the blood. Therefore a group was made of people belonging to group 3 for sea-fish, but to a different group for freshwater fish. This group was statistically compared with a combination of group 1 and 0 for sea-fish (table 3). It is obvious that also the consumption of much sea-fish causes a statistical significantly higher mercury content in blood than when fish is eaten very little or never.

Hair

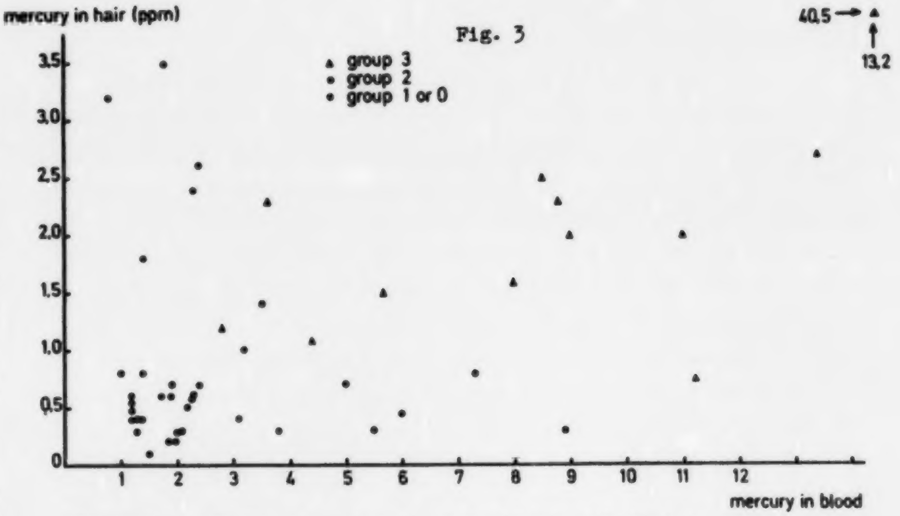
The frequency distribution of mercury in hair is given in fig. 2. Also in this case no normal distribution is found. The median value is 0.6 ppm (µg/g), while it can be calculated that:

68%	is	0 - 1 ppm
83%	is	0 - 2 ppm
92%	is	0 - 3 ppm
95%	is	0 - 4 ppm

Only 4 values were higher than 4 ppm. Of these probably 3 values (resp. 12, 16 and ± 200 ppm) have nothing to do with fish consumption, but can be caused by external contamination (use of shampoo containing mercury). These values have been omitted from the calculations. One value of 13.2 ppm belongs to the same person having a very high consumption of both sea- and freshwater fish and a blood content of 40.5 ppb.

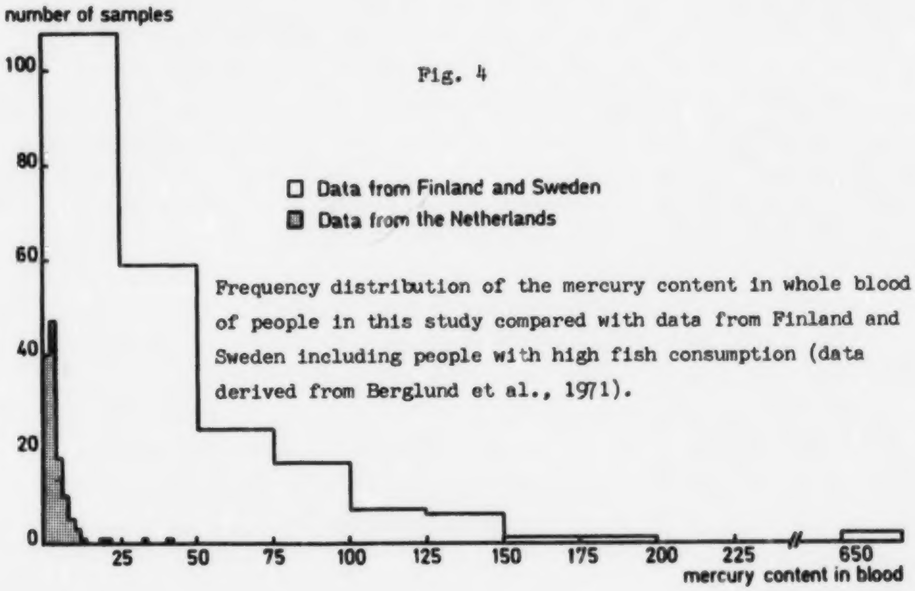
In table 4 the results of the mercury content in hair are given in relation to fish consumption (sea- and freshwater fish taken together). Also the statistical evaluation of the logarithmized values is shown. From this table it is obvious that hair of people with a high fish consumption contains more mercury than that of people eating less fish. Of the former group 94% has a mercury content of 1 ppm or higher contrary to 18% for the latter groups. Between the groups that eat fish at least once a month, at least once a year or never no differences exist in mercury content of hair.

Fig. 3



Comparison of the mercury content in blood with that in hair in 47 samples. Group 3 consists of people eating fish at least once a week, group 2 at least once a month, group 1 at least once a year and group 0 never.

Fig. 4



Frequency distribution of the mercury content in whole blood of people in this study compared with data from Finland and Sweden including people with high fish consumption (data derived from Berglund et al., 1971).

Correlation between blood and hair

According to Japanese and Swedish studies (Berglund et al., 1971) a correlation exists between mercury content in blood and hair. From 47 persons in our study samples of both blood and hair were obtained. The results for the mercury content are represented in fig.3 to study whether a correlation could be found. From this figure it is obvious that no clear correlation is present between values of mercury in blood and hair. Although a slight correlation was found by using Kendall's rank correlation test, this was caused mainly by the values of the people with a high fish consumption. Among people eating less fish sometimes relatively high values in blood are combined with low values in hair, but also the opposite is found.

For the whole group with a mean value of 1.3 ppm in hair and 4.7 ppb in blood a factor of about 280, but showing a large range, could be calculated. This is of the same order of magnitude as the factor of about 250 found by Berglund et al. (1971).

Discussion

Although the material selected for this study does not constitute a random test sample of the Dutch population, a number of valuable conclusions can be drawn. There is a clear correlation between fish consumption and mercury content in whole blood. This applies especially for people that according to their own statement consumed much sea- and freshwater fish. Some of them were even eating fish twice a day.

The mercury content in blood was less than 10 ppb in 94% of the people. In general the increase caused by fish consumption amounted to a few ppb's only. The mercury content in hair also showed a relation with (high) fish consumption. However because there was only a slight correlation between mercury in blood and hair (except for people with high fish consumption) determination of mercury in hair can not be used for the estimation of the mercury body burden of the Dutch population. Regarding to the mercury content of hair possibly other factors (like external contamination) play a role.

Although an obvious correlation was found for mercury in blood and fish consumption, the values obtained in this study are very low compared to values obtained in other countries. In the Swedish report of an expert group (1971) a frequency distribution is given of the mercury content in blood cells of people without clinical symptoms but with a high fish consumption . These data have been modified to whole blood (by dividing them by a factor 2) and in fig.4 they are compared with our results. It can be concluded that 98% of our data fall within the lowest group of the Swedish frequency distribution.

Recently a study was published (Skerfving, 1974) in which mercury levels in blood and hair of Swedish people consuming contaminated fish were compared with the health status. Clinical observation of people with mercury contents in blood of up till 200 ppb and in one case even 650 ppb did not show any abnormalities that could be ascribed to (methyl)mercury intoxication. Comparing our data with those mentioned in Berglund et al. (1971) the conclusion can be drawn that the mercury content in blood and hair in our study was relatively low or of the same order of magnitude as found in other studies for a normal population.

The preliminary results of a study in which 1200 blood samples of 18 year old Dutch men were analysed, show even a lower median value. In this case no data about fish consumption are known. It can be concluded that as yet it is not necessary to limit fish consumption in the Netherlands.

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DISCUSSION

CLEMENTE (Italy)

1. Could you please say what were the average Hg concentrations in the fish eaten by the Dutch population?

2. Were the hair samples washed carefully before analysis? If so it is hard to attribute the high Hg content in the hair to contamination by shampoos containing Hg. Hg in shampoos may in fact cause external contamination in the hair but this would surely be eliminated by proper rinsing.

DEN TONKELAAR (Netherlands)

1. The average Hg concentration in sea fish is approximately 0.1 to 0.3 ppm in freshwater fish approximately 0.5 ppm, occasionally higher, up to 1 to 2 ppm.

2. The hair was thoroughly washed before the analysis. Because hair contains many S-containing proteins it probably forms chemical bonds with the mercury contained in some shampoos which is therefore not eliminated by rinsing.

Van der KREEK (Netherlands)

Shampoos contains Hg as a preservative, not only in the Netherlands, but in most other countries too. The concentration in the shampoo never exceeds 30 ppb. I wonder if it is possible that this 30 ppb can lead to a Hg content of the hair of 200 ppm, the case you mentioned in your introduction.

DEN TONKELAAR (Netherlands)

The Hg concentration in shampoo is low, but our analysis mainly concentrated on the ends of the hair.

The high levels were found among girls with very long hair. In these cases the ends were perhaps as much as four years old and thus have been washed very frequently. In view of the fact that Hg can form chemical bonds with hair such a high level could be possible after a number of years.

DENNIS (Canada)

In a Saskatchewan survey it was found that recency of fish eating (fresh water fish within two weeks of sampling) was more closely correlated to higher blood mercury levels than frequency of fish eating.

In the same Saskatchewan survey involving 679 blood samples, month of sampling was a significant variable, as the fishing lakes are only free of ice for some 4 - 5 months of the year; during these months blood mercury levels are significantly higher and so is the consumption of fresh-water fish.

DEN TONKELAAR (Netherlands)

We did not consider this point in our study. Most of the blood and hair samples were collected during the summer and autumn. Sea fish is eaten throughout the year in the Netherlands, and freshwater fish mainly in summer (roughly up to October). Since the Hg found in fish is usually in the form of methylmercury, which has a fairly long half-life in man (approximately 70 days), I feel that the season in which most fish is eaten cannot have a particularly great influence on the results of measurements of mercury levels in the blood and hair.

BERNSTEIN (Canada)

1. Were whole strands of hair collected for analysis, and if so, were any of the hair samples with high mercury levels segmented to compare levels in proximal and distal segments?

2. In reference to one of the former queries from the audience, could not exogenous mercuriferous substances applied to hair bind quite firmly to hair protein and therefore be difficult to remove by washing the hair sample?

DEN TONKELAAR (Netherlands)

1. In most cases the ends of the hair were collected and in no case were whole strands analysed.

However, in one case a girl was found to have 200 ppm Hg in the ends of her hair. Individual hairs from her neck were later analysed and found to contain 2.3 ppm.

2. I fully agree with this observation.

SANTARONI (Italy)

I should like to know what the author thinks about the possible value of measuring levels of Hg contamination from the food intake of representative population groups and the amounts of Hg inhaled, given that blood levels are too variable in time, according to what our Canadian friend said, whilst measurements of hair can, as you said yourself, be affected by contamination from extraneous agents which are not necessarily metabolized.

DEN TONKELAAR (Netherlands)

Mercury levels in various food stuffs should certainly be determined so that an estimate of daily Hg intake may be made. On the other hand, according to English data, average daily intake can also be estimated from the mercury content of the blood.

THE CELL CULTURE AS TEST SYSTEM IN APPLIED ENVIRONMENTAL HYGIENE

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Düsseldorf, BRD**

ABSTRACT

The results of epidemiological studies in the field of environmental hygiene are the basis for controlled experimental research to identify the relevant effective factors. Human beings can only be used as test persons under certain circumstances and with special safety precautions, to test the biological effect of these substances singly and in combination. The laboratory animal and the cell culture are the appropriate test systems. The cell culture test system has been developed to such an extent that it is suitable for examining both the acute and the chronic biological effects of foreign matter from the environment. The harmfulness of a substance in vitro indicates its harmfulness also in vivo. The laboratory animal and the cell-culture are closely connected with regard to method and validity of results.

The object of the research is the use of the two test systems as screening processes for rapid assessment of environmental pollutants (toxicity, carcinogenicity). It is intended that these processes contribute to the elucidation of epidemiological findings and serve to facilitate decisions on preventative measures in the field of environmental protection.

Results will be presented which have been obtained with the cell culture test system. The substances tested were atmospheric dust (extracts, components) from immissions in industrial conglomerations and urban and rural areas.

In pharmacological and toxicological research the test system cell culture has attained a firm place. Quite apart from the considerable saving of time and cost, this system offers the following advantages: 1. the possibility of studying events on the cellular level; 2. the use of homogeneous cell populations; 3. experimental conditiond which are easy to survey, i.e. defined, quantifiable and reproducible; 4. due to the merits stated under points 1 and 2 a good possibility for statistical affirmation. The limits of this method should, however, also be pointed out: 1. lack of those integration mechanisms (humoral, nerval) to which the cell is subjected in the whole organism; 2. lack of metabolization of offered substances in the sense of their activation or detoxification; 3. difficult and limited extrapolation of results on animals or man, though correlations do exist. As an example, studies in the field of pneumoconiosis research should be cited which showed a good correspondence of the relations between the frequency of silicosis of mine dusts, their fibrinogenic action in vivo, and their cytoxicity in vitro (1,2).

In slides the test objects, i.e. diploid and permanent proliferating and nonproliferating cells in vitro and the test parameters like cell morphology, physiology, functions, and specific faculties are presented (figs. 1 to 5).

Alveolar macrophages are a test system providing a link between in vivo- and in vitro-conditions. The exposition can take place in vivo as well as in vitro, while for the evaluation of the effects the advantages of the in vitro system can be used. Alveolar macrophages play a decisive role in the defense of the lung against particulate air pollutants. As it is possible to cultivate them as a homogeneous cell population, the cell culture offers the opportunity for investigations which are non-existent in vivo. As an instance, experiments were conducted by BRUCH et al. regarding the question whether the faculty of macrophages to metabolize benzo(a)-pyrene is influenced by inhaled lead (3). After exposition to a lead aerosol, the alveolar macrophages of the experimental animals were obtained by pulmonary lavage, cultivated in vitro, and incubated with benzo(a)pyrene. It was noted that the quantity of benzo(a)pyrene metabolized by the alveolar macrophages of lead exposed animals is

CELL TOXICOLOGY

I. Test objects:
cells in vitro

II. Parameters:
- a) morphology
- b) physiology
- c) functions
- d) special functions or abilities

Fig. 1

CELL TOXICOLOGY

I. Test objects in vitro:

a) diploid cells	(mammalian,	proliferating
b) permanent cells	including human)	
c) macrophages	(mammalian,	non-proliferating (without stimulation)
d) lymphocytes	including human)	

Fig. 2

CELL TOXICOLOGY

II. Parameters in vitro:

a) Cell morphology:

1. Form and structures: nuclear and nucleolar structure
   endoplasmic reticulum
   lysosomes
   lipid droplets
   vacuoles
   degenerating forms
2. Morphokinetics: motility
   membrane activity
   plasma flow
   necrobiosis

Fig. 3

CELL TOXICOLOGY

II. Parameters in vitro:

b) Cell physiology:

1. Energy metabolism:	lactate production glucose consumption O_2-consumption ATP/ADP quotient
2. Structure metabolism:	RNA synthesis protein synthesis

Fig. 4

CELL TOXICOLOGY

II. Parameters in vitro:

c) Cellular functions:

1. Reproduction or cell kinetics:	cell number mitotic rate plating efficiency generation analysis (DNA metabolism)
2. Permeability:	enzyme release dye uptake
3. Phagocytosis/Pinocytosis	

Fig. 5

only a fraction of the control values proving impairment of this specific cellular function. These experiments confirmed earlier findings concerning the disturbance of benzo(a)pyrene metabolism of alveolar macrophages following in vitro exposition to lead or ambient dusts (4). In the course of the investigations on the cytotoxicity of particulate air pollutants it was found that in this case dose-effect relationships must be regarded from the point of view of the hit theory. Microkinematographic observations have shown that one lead particle is capable of causing lysis of a cell (1 particle + 1 cell = 1 hit = cell death). As regards the lung, one should also proceed from the assumption that punctual damages occur during the inhalation of particulate matter, which, upon accumulation, can be of pathogenetic significance. BINGHAM and colleagues demonstrated a distinct reduction in the number of alveolar macrophages which could be obtained by lung washings from rats having inhaled lead for a longer period of time (5). Moreover, BRUCH et al. detected a reduced lung clearance in lead exposed experimental animals (6).

Alveolar macrophages are differentiated postmitotic cells which also do not multiply under normal tissue culture conditions in vitro. However, with the use of diploid fibroblasts and permanent cells the essential property of the cell to proliferate can be tested. Dose-response relationships concerning one substance can be established and also different substances can be compared with each other. To give an example, the influence on cell growth and metabolism of the environmentally relevant heavy metals lead, cadmium, mercury, zinc, and tin was studied in a mouse fibroblast line and clear concentration dependencies were found. Different doses of these metals cause a 50% reduction of replication with Sn showing the lowest and Cd a 1000-fold higher cytotoxicity, while Pb, Zn, and Hg are in between. Thus in this model a Toxicological rank order could be established (7).

Fibrous materials gain increasing importance in the context of environmental toxicology. In animal tests it was proved that besides asbestos also glass fibres induce the formation of foreign body granulomas and malignant tumours (8,9). In cell cultures fibriform material causes a specific incorporation mechanism which is dependent on form and length of the fibre (4). The delayed and incomplete phagocytosis leads to a localized leakiness of the cell with persistent but compensated loss of enzymes and increased energy metabolism. The engagement between the cell and the fibre results in a chronic irritation which is being discussed as one of the causes of tumour formation. In this context it is of interest that fibrous material induces the formation of giant cells in vivo as well as in vitro. In vitro it was proved that the polycarocytes caused by asbestos or glass fibres are the product of cellular fusion (10).

Cellkinetic and biochemical studies contribute to the elucidation of the modes of action. Such fundamental investigations are often only possible with the aid of cell cultures. Following assessment of the relations between the results obtained in cell culture as a screening system for the evaluation of the toxicity of novel environmental noxious substances.

It has been known for many years that cells in vitro can be transformed into tumour cells by a number of DNA- and RNA-viruses. The first report concerning the oncogenic transformation in vitro by chemicals was given by BERWALD and SACHS in 1963 (11). In the meantime, tissue culture cells are being used more and more in tumour research for the evaluation of the oncogenic capacity of chemical substances and their modes of action (12,13). This test system offers several advantages as compared to the whole animal. The starting point of cancer is the malignant change of the cell. The cell culture makes possible the direst study of this event with regard to morphological and functional aspects.

The test objects and parameters for oncogenic transformation in vitro are presented in figs. 6 and 7.

CELL TOXICOLOGY

II. Parameters in vitro:
  d) Specific functions or abilities
    1. Drug metabolism
    2. Stimulation e.g. by PHA (lymphocytes)
    3. Bactericidal action, virus susceptibility (interferon production)
    4. Migration of cells in the electric field

Fig. 6

In the context of air pollution research, the question is of paramount importance whether particulate air pollutants or their extracts are capable of transforming cells in vitro. In the literature only a few papers are known under this aspect. Special mention should be made of the studies executed by FREEDMAN and collaborators on the transforming action of extracts of "city smog" (14). The authors

ONCOGENIC TRANSFORMATION IN VITRO

I. **Test systems**:

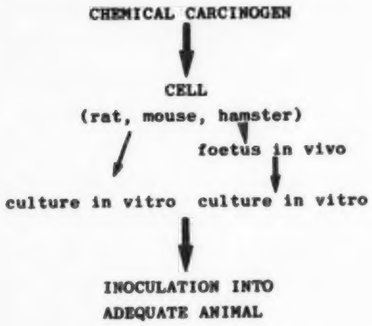

Fig. 7

could demonstrate that the city smog extracts contained one or more factors with a 600-fold oncogenic capacity than pure benzo(a)pyrene. As the decisive proof for transformation in vitro to have occurred the capability of the cells to form tumours in an adequate animal was considered.

The difficulty that some carcinogenic substances have to be activated or metabolised in vivo limits the use of in vitro/in vivo experiments

ONCOGENIC TRANSFORMATION IN VITRO

II. **Parameters:**

multilayered growth
(loss of contact inhibition)

loss of specific growth patterns

increased multiplication
(decreased generation time)

increased plating efficiency

altered metabolic behaviour
(increased glycolysis)

altered karyotype

unlimited proliferative capacity in vitro

tumour formation after inoculation
in adequate animal

Fig. 8

like the one described above. DI PAOLO et al. developed an elegant test system combining in vivo- and in vitro-methods in such a manner that exposition occurs in vivo while the transformation takes place in vitro (15). To date this test system has yielded the expected results in 12 carcinogenic substances. The new test promises to become a suitable screening method for the assessment of the carcinogenicity of environmental pollutants or their metabolites.

The investigations regarding the toxicity and oncogenicity of environmental chemicals in vitro demonstrate the increasing significance of the cell culture as a test system in applied research. It is the aim of these studies to employ the cell culture, also in connection with the experimental animal, as a screening system for the rapid detection of noxious substances in the environment.

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DISKUSSION

BIANCO (Italien)

Ich habe drei Fragen:

- Welche Technik wurde bei den Inhalationsbelastungsversuchen benutzt?

- Haben Sie die Konzentration der verschiedenen Partikel in der für die inhalative Belastung verwandten Luft bestimmt?

- Haben Sie die Korngrössenverteilung und die geometrische Standardabweichung der Partikel ermittelt?

BECK (Bundesrepublik Deutschland)

Die zitierten Tierversuche wurden von Dr. Bruch und Mitarbeitern (Düsseldorf, BRD) durchgeführt.

BRUCH (Bundesrepublik Deutschland)

Die Technik der Versuchsdurchführung wurde in dem Vortrag "Ueber lokale Effekte inhalierter Bleiverbindungen auf die Lunge" beschrieben. Das Bleiaerosol wurde durch Kondensation von verdampftem Bleioxid erzeugt. Die Bestimmung der Konzentration erfolgte an Membranfiltern mit Hilfe der Atomabsorptionsspektrometrie. Die Grösse der Teilchen war kleiner als 0,5 /um.

HINE (U.S.A.)

Haben Sie Schleimhautzellen oder Lymphocyten von Personen untersucht, die Expositionen gegenüber Cadmium, alkylierenden Agentien oder schwer verunreinigter Stadtluft aufwiessen?

BECK (Bundesrepublik Deutschland)

Solche Untersuchungen sind geplant. Als vorbereitende Versuche haben wir Zellen von Tieren untersucht, die über längere Zeit atmosphärischen Feinstaub einzeln oder in Kombination mit Fremdgasen (SO_2/ NO_2) inhaliert hatten. Wir stellten fest, dass die Zahl der aus der Lunge gespülten Makrophagen dieser Tiere reduziert und dass spezifische Funktionen der Alveolarmakrophagen (z.B. drug metabolism) beeinträchtigt waren. Von Dr. Manojlovic (Düsseldorf, BRD) wurden zur Prüfung der individuellen Disposition Lymphozyten von Versuchspersonen in vitro mit Quarz und PHA inkubiert und anschliessend Mitose- sowie Transformationsrate der Lymphozyten untersucht.

ZIELHUIS (Niederlande)

Wie erklärt Herr Dr. Beck die von Cooper (USA) veröffentlichten Angaben, aus denen sich kein Hinweis einer erhöhten Sterblichkeit infolge von Erkrankungen der Atemwege ergibt, und was sagt er zu der allgemeinen Erfahrung, dass es keine erhöhte Sterblichkeit durch Erkrankungen der Atemwege gibt, im Blickwinkel seiner Versuche und insbesondere der "Treffer-Theorie"?

Mit anderen Worten, wie vereinbart er seine Versuche mit dem weiten Erfahrungsschatz der Humanmedizin?

BECK (Bundesrepublik Deutschland)

Ziel des Übersichtsreferates über die Verwendung der Zellkultur in der Umweltmedizin war, den Stellenwert dieses Testsystems aufzuzeigen und bestehende Korrelationen zwischen Ergebnissen von in vivo- und in vitro-Untersuchungen darzustellen. Die Untersuchungen über die Wirkung von partikelförmigem Blei in der Zellkultur und im Tierversuch sind also hier in diesem Zusammenhang zu sehen.

Im Falle des Bleis wird besonders von Toxikologen die Meinung vertreten, dass erst der Nachweis einer chronischen Toxizität gesundheitspolitische Massnahmen rechtfertigt, Präventivmedizinern genügt für solche Massnahmen dagegen bereits der begründete Verdacht für eine Gesundheitsgefährdung vor allem der Personen, die durch Krankheit oder Alter in ihrer Widerstandskraft geschwächt sind, besonders, wenn berücksichtigt wird, dass Blei innerhalb der "toxischen Gesamtsituation" nur einen Faktor unter vielen darstellt.

In der Zellkultur ist bei Applikation von partikelförmigen Stoffen die Dosis-Wirkungs-Beziehung unter dem Gesichtspunkt der Treffer-Theorie zu betrachten. Dies gilt letztlich auch für die Zellen in der Lunge nach Inhalation partikelförmiger Stoffe. Jeder aktive oder passive Kontakt eines Staubteilchens mit der Zelle ist als ein Treffer zu betrachten. Jeder Treffer wird eine Reaktion der Zelle auslösen. Die Summation der Einzelereignisse kann pathogenetisch bedeutungsvoll sein.

DANIEL (G.B.)

Gewebekulturen bieten eine Möglichkeit, Verbindungen auf ihr toxikologisches Potential hin zu untersuchen. Sie stellen ferner eine verfeinerte Methode dar, die sich zur tiefergehenden Untersuchung des Wirkungsmechanismus von Verbindungen auf der Zellebene eignet. Ich möchte den Referenten fragen, welche biochemischen Parameter seiner Meinung nach als die empfindlichsten Indikatoren toxischer Wirkung anzusehen sind. Ich spreche nicht von histochemischen Verfahren; mir geht es darum, mehr quantitative Angaben zu sammeln, so dass die Dosis-Wirkungs-Beziehung festgestellt und damit die Beurteilung des jeweiligen Risikos erleichtert werden kann.

BECK (Bundesrepublik Deutschland)

Auch wir sind der Meinung, dass das Testsystem Zellkultur inzwischen soweit ausgereift ist, dass es sich zur Prüfung sowohl der akuten als auch chronischen biologischen Wirkung von Fremdstoffen der Umwelt eignet. Bezüglich Ihrer Frage, welche biochemischen Parameter unserer Meinung nach als die empfindlichsten Indikatoren einer toxischen Wirkung anzusehen sind, möchte ich in erster Linie die Zellpermeabilität nennen. Ein intakter Zellstoffwechsel ist die Voraussetzung zur Aufrechterhaltung einer physiologischen Permeabilität. Wird Z.B. der Energiestoffwechsel durch toxische Einwirkung gehemmt, so ist ein Enzymverlust die Folge. Eine Störung der Zellpermeabilität wird aber nicht nur durch einen toxischen Eingriff in den Energiestoffwechsel verursacht, sondern ebenso durch direkte Wirkung auf die äussere Zellmembran. Als Kriterium für den Nachweis einer erhöhten Permeabilität wird von uns die Freisetzung intrazellulärer Enzyme herangezogen, gemessen an ihrer Aktivität im überstehenden Nährmedium. Die extrazelluläre Enzymaktivität ist ein repräsentativer Indikator für die Permeabilität der Zelle. Dabei sollte besonders hervorgehoben werden, dass wegen der geringen benötigten Probenmenge kontinuierliche Messungen der Enzymaktivität und parallel dazu morphologische Untersuchungen der Zellen im Versuchsablauf möglich sind. Als weiterer Indikator für eine Störung der Permeabilität dient die Aufnahme saurer bzw. der Verlust fluorochromer Farbstoffe. Die Fluorochromasie kann quantitativ erfasst werden, entweder mit Hilfe der Impulszytophotometrie oder aber durch Messung der Emissionsenergie des in den Überstand freigesetzten Farbstoffes.

Als weitere empfindliche Parameter für eine toxische Wirkung auf zellulärer Ebene eignen sich der ATP/ADP-Quotient und der O_2-Verbrauch (kontinuierliche polarographische Messung) als Ausdruck des Energiestoffwechsels. Von besonderem Wert als Kriterium für eine Schadstoffwirkung ist schliesslich die Aufzeichnung des Habitus und der Motilität der Zellen im Zeitrafferfilm. Auf diese Weise lässt sich das Verhalten von Zellen (Vermehrung und Motilität) kontinuierlich registrieren und quantitativ auswerten.

EPIDEMIOLOGY OF PESTICIDE AND METAL RESIDUES

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(Editorial Note: Paper presented by J.A. Santolucito)

ABSTRACT

Studies relating to the epidemiology of pesticide and metal residues involve the formulation of appropriate research models. These models must be formulated in order to quantitate the impact of exposures involving multiple routes to man and to test specific hypothesis relating these pollutants to adverse health effects.

Crucial to all of these studies is the investigation of human body burdens of these residues using both the general population or selected subgroups at greater risk either from occupational or geographic exposure.

The studies we have conducted have been designed to address a number of questions in several areas. The first series of investigations was aimed at determining which tissues are most suitable for monitoring body burdens of the residues in human populations. Thus blood, urine, hair, feces and placenta were analyzed for pesticide, trace metal and synthetic organic residues. In addition, multiple tissue sets were collected at autopsy and analyzed.

The second series of studies was designed to test specific hypothesis relating disease risk to pollutant levels. Typical of the questions investigated were the relationship between metals and organic burdens and cardiovascular disease and toxemia of pregnancy.

The final phase of our research efforts focused on linking tissue burdens with refined laboratory indices of health impairment. Examples of these studies include the effects of metals, organics and pesticides on placental enzyme activity, hematological indices and detoxification mechanisms along with changes in other critical clinical parameters.

This linkage of clinical and laboratory approaches to a coordinated epidemiological system allows not only a current assessment of risk in these populations but, in addition, a more predictive posture as new pollutant problems arise.

To date these studies have yielded interesting preliminary results. Whereas easily collected specimens such as hair and blood have proved utility in estimation of environmental exposure, these tissues cannot, as yet, be considered a good quantitative indicator of many body pollutant burdens. Studies of occupationally exposed groups or groups of individuals with clinically evident disease have failed to clearly implicate pollutant burdens with either disease production or aggravation although, in general, the blood levels of these individuals do reflect increased exposure to pollutants. Studies of multiple tissue sets collected at autopsy are encouraging inasmuch as it appears these tissues will provide useful information about the pollutant burden for constructing predictive models and, in addition, provide flashback capabilities.

1. Introduction

Studies relating to the epidemiology of pesticide and metal residues involves the formulation of appropriate research models. These models must be formulated in order to quantitate the impact of exposures involving multiple routes to man and to test specific hypothesis relating these pollutants to adverse health effects.

Crucial to all of these studies is the investigation of human body burdens of these residues using both the general population of selected subgroups at greater risk either from occupational or geographic exposure.

To those of us concerned with health effects, clear definition of the linkage between pollutant burdens and biological response becomes a matter of great importance. This is particularly true because human pollutant burdens can elicit a broad spectrum of biological responses that are not clearly separable. Specifically, a pollutant burden can exist with no associated physiological changes or it can be associated with physiological changes that are sentinels of disease and have a strong association with morbidity or mortality.

2. Approach

In addressing itself to these problems, our group has currently mounted projects in several areas. The first series of investigations is designed to determine what tissues are most suitable for monitoring pollutant burdens in large populations. Thus, blood, urine, hair, excreta, and placenta are assayed for selected trace metals and synthetic organic compounds. In addition, multiple tissue sets are being collected at autopsy and analyzed. Whenever possible, study groups are chosen because of their exposure to an appropriate pollutant gradient. Another aspect of this first series of studies is the establishment of a national tissue bank to provide flashback capabilities as new problems emerge, and to allow accurate assessment of tissue distribution profiles of the various pollutants in the human population.

The second series of studies we are conducting is designed to test specific hypotheses that relate disease risk and pollutant levels. Typical of the questions investigated are such problems as the relationship of specific metals to indices of cardiovascular disease and the transplacental transfer of metals and toxemia or pregnancy. The rationale is to correlate pollutant burdens with known disease symptoms and assess their role in disease production or aggravation. Focusing on selectively vulnerable or occupationally exposed groups permits direct assessment without the large dilution factors operative in general population studies.

The final phase of our research efforts is focused on linking pollutant burdens with refined laboratory indices of health impairment. Specific examples of these studies include the effects of metals and organic compounds on profiles of placental enzyme activity and pollutant-induced changes in detoxification mechanisms.

Selection of tissues for evaluation of pollutant burdens is currently under active investigation by our group. A number of studies have indicated a correlation between trace metal content in hair and exposure; an example is shown in Table 1. As can be seen, levels of arsenic, cadmium, and lead in hair closely follow the exposure gradient, whereas zinc and copper levels do not. The latter two metals, it should be recalled, are essential to man, and homeostatic mechanisms for their metabolism are known to exist.

Studies of occupationally exposed groups such as men exposed to cadmium dust from superphosphate production have established the utility of blood as an index of exposure. In these workers (Table 2), plasma levels of cadmium and zinc were elevated in both the intermediate- and high-exposure groups. By contrast, urine levels failed to show any correlation with exposure.

Attention has been focused on organic pollutants as well. By examination of the plasma of over 700 healthy individuals, polychlorinated biphenyl (PCB) residues, ranging in concentrations up to 29 parts per billion (ppb), could be found in 43%. In addition, residues of pp'DDDT and DDE were found in 84% and 63%, respectively, of the individuals tested. Interactions involving ethnic differences and ethnic residence were noted for most residues as well. Residues of polychlorinated biphenyl were found more frequently and at higher levels in white, urban residents and rare (4.1%) in rural blacks (Table 3). In refuse workers potentially exposed to polychlorinated biphenyl from incineration of numerous materials, residues of PCB were identified in the plasma of 81% (32 of 37 workers) while only 11% (6 of 54) of the control group showed abnormal evidence of these residues. Median levels of PCB for detectable samples were 2.6 ppb (exposed), 3.7 ppb (controls), and maximum levels in plasma were 14.1 ppb (exposed) and 20.2 ppb in the controls. Scalp hair in this study was found to be of no utility in estimating exposure to PCB. (Table 4)

TABLE I. DISTRIBUTION OF TRACE METAL LEVELS IN HAIR

Metal	Exposure* Ranking (City)	Arithmetic Mean, ppm†	Standard Deviation, ppm	Sample Size
Arsenic	5	10.6	7.00	31
	4	5.2	6.00	16
	3	1.7	1.48	32
	2	0.8	0.33	13
	1	0.4	0.26	28
Cadmium	5	3.5	4.94	45
	4	2.0	1.54	85
	3	1.3	0.99	37
	2	1.3	1.30	21
	1	0.9	0.58	37
Copper	5	25.7	28.1	45
	4	15.3	7.5	37
	3	11.8	3.0	25
	2	12.6	6.0	21
	1	22.5	34.7	37
Lead	5	107.1	131.8	45
	4	44.3	49.3	25
	3	14.3	14.1	37
	2	12.1	11.4	21
	1	7.6	5.0	38
Zinc	5	154.0	33.7	45
	4	145.2	30.8	25
	3	156.6	26.0	37
	2	155.4	36.9	21
	1	154.2	32.5	38

*Exposure Ranking 5>4>3>2>1.
†Parts per million.

TABLE II. AVERAGE CADMIUM AND ZINC LEVELS IN URINE AND PLASMA OF WORKERS EXPOSED TO CADMIUM DUST

Exposure Category	Cadmium	Zinc
Plasma Concentration, μg/100 ml		
Low	0.42±0.7	54±32
Intermediate	2.7 ±2.7	136±68
High	1.9 ±2.0	104±64
Urine Concentration, μg/Liters		
Low	1.04	630±550
Intermediate	1.14	440±350
High	1.10	630±400

TABLE III. PLASMA RESIDUES OF SELECTED CHLORINATED HYDROCARBONS*

Race-Residence Grouping	Mean Plasma Residue			
	Total DDT, ppb†	Percent Measurable	Total PCB, ppb	Percent Measurable
Rural black	20.90	100	0.35	4.1
Urban black	12.50	98.3	1.97	49.3
Rural white	5.57	97.0	3.18	50.9
Urban white	4.71	99.0	2.35	55.7

*Sample taken among residents of Charleston County, South Carolina.
†Abbreviations: DDT, dichlorodiphenyl trichloroethane; ppb, parts per billion; PCB, polychlorinated biphenyl.

TABLE IV. PLASMA LEVELS OF POLYCHLORINATED BIPHENYLS IN REFUSE WORKERS AND CONTROLS

Group	No. of Observations	No. With Measurable Levels	% Measurable	Mean Levels, ppb*	Maximum Levels ppb
Control	54	6	11	3.7	20.2
Exposed refuse workers	37	32	81	2.6	14.1

*ppb - parts per billion

As can be seen from studies of this type, easily collected tissues, such as hair and blood, can be of utility in estimation of exposure. However, a great need exists to relate these levels of pollutants in the hair and blood to actual tissue burdens.

To begin this task, our group has conducted feasibility studies focused on trace metal analysis of multiple tissue sets collected at autopsy. Preliminary results have revealed some trends between the amounts of lead, copper, iron, cadmium, and zinc in scalp hair and the amounts in bone, kidney, liver, lung, and aorta tissue, but more data will be needed before rigorous, predictable relationships can be established.

When we have investigated the relationship between pollutant burdens and selected populations at risk or subjects with disease, the problems have become even more manifest.

Studies in maternal-fetal tissues have provided evidence for accumulation and transplacental transfer of metals. Analysis of these sets (Table 5) demonstrated that placental cadmium levels were one to two times the levels found in maternal or cord blood. It was observed also that erythrocyte cadmium levels were roughly three to five times plasma cadmium levels, and that maternal erythrocyte cadmium levels were somewhat higher (27%) than those noted for the fetus. In addition, cadmium levels in the meconium were very similar to those found in maternal blood. The data on lead is only partially complete, but its transfer and accumulation is already known. The problems relating mercury to fetal Minamata disease are familiar.

TABLE V. RELATIONSHIPS BETWEEN CADMIUM AND LEAD LEVELS OBSERVED IN TISSUES FROM MOTHERS AND NEWBORN CHILDREN

	Arithmetic Mean			
Tissue	No. of Pairs	Cadmium (μg/100 gm)	Lead (μg/100 gm)	Ratio, Lead to Cadmium
Placenta	100	7.3	96	13.2
Cord Plasma	100	1.4	...	...
Maternal plasma	100	1.5	...	...
Placenta	25	6.8	...	...
Maternal scalp hair	25	374.0	2,933	7.8
Maternal perineal hair	25	309.0	1,236	4.0
Maternal whole blood	25	3.7	33	8.9
Maternal erythrocytes	25	7.1	58	8.2
Cord blood	25	4.6	44	9.6
Cord erythrocytes	25	5.6	46	8.2
Merconium	25	8.6	26	3.0

These data are thus clearly indicative of transplacental transfer of cadmium, yet metal levels could not be causally linked to increased blood pressure or decreased weight of the newborn infant, nor could they be shown to increase in toxemia of pregnancy as has been alluded to by other workers.

Studies in persons who died of cardiovascular disease demonstrated no consistent elevation in renal cadmium concentrations, and the apparent elevations in renal cadmium concentrations previously observed may have been occasioned by the confounding effects of the widely varying renal cadmium levels noted in cancer deaths.

Similarly, although elevated levels of plasma cadmium were observed in superphosphate workers, these elevated levels could not be linked to changes in hemoglobin levels, hematocrit readings, blood pressure, and cholesterol or serum lipid levels in the exposed groups.

Thus, it is clear that the mere presence of a pollutant in tissue is not always sufficient evidence of disease potential, and one must be careful in ascribing the cause of disease to pollutants fortuitously associated with clinical symptoms. Pharmacological experience has taught us that the toxicity of an agent is not always expressed at the site of maximum concentration, and it may be manifested only by subtle changes. Thus, our final series of studies currently underway is designed to link the effects of pollutant burdens with refined laboratory indices of health impairment. These studies are designed to answer several questions. First, what subtle biochemical changes occur in individuals under certain pollutant burden stresses? Second, what changes in readily assayable tissue can correlate or quantify these effects? And finally, what is the dose-response relationship between pollutant burdens and changes in critical physiological and biochemical systems? These studies are recent in conception, but the preliminary results appear promising. One promising index currently under investigation utilizes the profiles of placental enzyme activity. This two-part project examines enzymes that may be induced or altered by specific pollutants such as hydroxylases and metalloenzymes and, in addition, other enzymes that are rate-limiting for selected metabolic cycles.

Assessment of changes in nonenzymatic areas is also necessary. Thus, we examine protein profiles and critical substrate levels in blood and other tissues as well. Bioenergetic and immunologic systems may also reflect effects of pollutant burden. In our laboratories, preliminary studies are underway, and attention is being focused on development of mitochondrial and microsomal assays that reflect such pollutant burden sensitivities.

Clearly, the problems encountered in linkage of refined laboratory indices to pollutant burdens are even more complicated than those encountered in other studies. Inhibition of any enzyme by a pollutant is an effect worthy of note, but relation of this effect to disease production in man requires careful documentation. Similarly, direct or indirect changes induced by exposures to pollutants in areas known to be sentinels of disease must be carefully evaluated. Adverse effects must be separated from normal or even beneficial changes and dose response is a prime concern. Transient changes in any dimension can have a cause far removed from pollutant effects themselves, and clear patterns in controlled studies must be obtained. It is our belief, however, that these biochemical and physiological indices hold great hope for the future. Factors such as these are applicable to living human populations in a practical sense and have the advantage of being relatively inexpensive to assay and are becoming very well standardized.

In the design and implementation of all these studies, a critical need exists for multiple tissue analysis. In response to this need, we have initiated on a pilot scale a national tissue bank designed to permit present and future evaluation of pollutant levels. By accumulation of tissues from humans at autopsy, we can assess the relationship between pollutant burdens in various tissues. Similarly, this tissue bank will permit collection of sufficient tissues from cases of verified disease and allow more comprehensive investigation of correlations between patterns of pollutant burdens and these disorders. In addition, this tissue bank will provide flashback capabilities should, as almost certainly will be the case, new pollutant problems arise in the future.

This discussion has given only a brief overview of our efforts in the area of assessing and characterizing effects of pollutant burdens, but we feel it represents an integrated approach to the solution of these complex problems.

DISCUSSION

SILBERGELD (U.S.A.)

With reference to the first slide, showing levels of various metals, is there any relationship, direct or inverse, between the lead and zinc levels observed in your sampled population?

SANTOLUCITO (U.S.A.)

The data presented were preliminary and because of the small sample size inferences on a relationship between lead and zinc levels were not made.

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STORAGE MAP OF ORGANO-CHLORINE COMPOUNDS (OCC) IN HUMANS
EPIDEMIOLOGICAL DEDUCTIONS FOR FURTHER MONITORING

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ABSTRACT

It is characteristic of living organisms to carry the foreign compounds which enter them through continuous cycles of activity like their own components. Some of the compounds on the environment accumulate to some extent in the animal body. This storage constitutes a dynamic process.

In the framework of a joint programme WHO-International Agency for Research on Cancer and the Hebrew University-Hadassah Medical School, Department of Occupational Health, Jerusalem, the OCI storage in humans has been investigated in various populations in Africa, Asia and South America.

Data obtained in these studies led to the conclusion that the age group 25-44 years stored the highest amount of OCI in both sexes for most populations. Since after the age of 45 years a change in the amount of OCI stored was observed, it is suggested that the age group of 25-44 years may characterize the OCI storage level of a community and can be compared with the mean storage level of stillborns in order to assess the increase in man's OCI impregnation from his fetal life.

The paper presents a map of the storage level of OCI in hu-ans throughout the world according to data reported in literature. estions for the way of further monitoring are presented.

The importance of the assessment of the OCC body load in the general population and in occupationally exposed workers evolves from the circumstances of continuous pollution of the environment with remanent, biologically active compounds.

The OCC map we intend to draw has this justification. Moreover, it may constitute a reference point for further epidemiological studies.

A quarter of a century has passed since the first publication of DDT storage in man (Howell, 1949).

The study of OCC storage in the general population and occupationally exposed people, which began in the U.S., spread to Europe, Asia, and the other continents and has now reached great development in Japan.

From the data available on OCC storage, the following facts emerge:

1. The storage of OCC in man is a widespread phenomenon, in fact they are a current constituent of humans living in the second half of this century.

2. The storage of OCC is proportional to the degree of exposure, since occupationally exposed people store the highest levels (128).

3. The storage of OCI is higher in men, and there is a direct relation to age, starting with intrauterine life up to the age of 45 years. In some countries, (USA, Israel) this direct relationship exists for all the age groups, while in others a decrease in the storage level occurs after 45 years of age.

4. The geographical location of the groups samples seem to have an influence on OCI storage. The storage is higher in south and east Europe when compared with north and west Europe. The same applies when we compare Canada to the United States. The cause of these geographical differences may be local agriculture or sanitary practices.

5. The physiological state of the body (e.g. pregnancy), obesity, loss of weight and pathological conditions like liver disease (86), carcinoma (85), seem to influence the storage level of OCC in opposite directions. (The milk of obese women contains lower amounts of OCI than that of women with normal weight (Germany, DDR (70)).

6. Race also seems to influence the storage level. Davies (30) found higher concentration in Negroes of the United States. In South Africa, a lower level of OCI storage was reported by us in non-white people (127). It is possible that socio-economic conditions may explain such differences. In a study of OCI serum levels in a multiracial population, significant differences were found among the various ethnic groups: sera from Chinese contained the highest levels of p,p'-DDT and β-BHC. Koreans had the highest levels of Dieldrin, and people from triracial backgrounds had the highest levels of γ-BHC (64).

7. There is a bidirectional relation between OCI and some drugs which influence their storage level by activation or inversely by inhibition of enzymatic systems involved in the metabolization of these compounds. (Volunteers receiving diphenylhydantoin (31) as well as patients receiving phenobarbital and/or diphenylhydantoin (137) had a lower OCI storage level when compared to controls. In an OCI plant, one of the workers who took phenobarbital and diphenylhydantoin over 25 years for post traumatic epilepsy had no or trace amounts of DDT and DDE residues in his serum (73).

8. The banning of special OCC which reached too high a level in special areas (e.g. β-BHC in Japan) led to decrease in storage of the banned compound (β-BHC in Japan decreased 20-50% (89)).

9. The exposure of the general population to OCI occurs especially from contaminated food but also from household use (β-BHC residue was higher in the milk of non-farm women in Japan (9,114)). Mothers in non-agricultural families consume larger amounts of beef and milk daily, when compared to farm women (49).

10. The greatest exposure of the general population, non-occupationally exposed to OCC, is that of infants fed by mother's milk. Mother's milk has a higher OCC residue than cow's milk and in some regions the exposure is high enough to cause biological effects. (Inhibition of corticoids synthesis, gluconeogenic enzyme activity, and interference with calcium, vitamin D and sex hormone metabolism (7)). In Guatemala, OCI residue of mother's milk is 25-30 times the average level found in the USA, England, and Sweden (Löfroth, 76).

In conclusion, the epidemiological and analytical findings regarding the OCC storage in man provide, at this time, a reasonable basis for adopting a more uniform approach in assessing the storage levels of these compounds in the general population.

Accordingly, we propose:

1. The age group 25-45 years should constitute a reference group characteristic of the OCC storage of a community.

2. A uniform method for the entire analytical procedure of OCC assessment should be adopted.

3. The findings should be reported both as OCI in extracted lipids and in whole tissues or biological fluids.

4. The measurement of OCI residues in the human body should be considered as a component of a national program in public health, since it proved to be a valuable indicator of the exposure to OCC and consequently a basis for implementing suitable preventive programs.

Table 1. Storage of OCC in fat tissue of humans (ppm). North America

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCB	HCB	Year
Laug et al.	74	USA	5.3					1950
Hayes et al.	52	USA	11.7					1955
Dale & Quinby	29	USA	6.7	0.20	0.15			1961
Hoffman et al.	55	USA	10.3	0.57*	0.11			1964
Quinby et al.	100	USA	12.7					1961
Hoffman et al.	54	USA, Chicago	10.4	0.48*	0.14			1963
Hayes et al.	51	USA, New Orleans	10.3	0.60*	0.29			1965
Fisherova Bergerova et al.	43	USA	10.6		0.22			1966
Zavon et al.	143	USA	7.6		0.31			1964
Shafer & Campbell	108	USA	2-31					1964
Edmundson et al.	37	USA, Florida			0.22			1965
Morgan & Roan	82	USA, Arizona	6.5		0.14			1967
Davies & Edmundson	30	USA, Florida	12.4					1966
Curley et al.	24	USA, Atlanta	8.8	0.55	0.24			1969
Warnick	136	USA, Utah	9.0		0.20			1968
			7.2		0.15			1969
			5.3		0.15			1970
Price & Velch	98	USA, Michigan				>2.0		1972
Jobs, A.R.	59	USA				>2.0		1972
Birosand Walker	14	USA						1970
Durham et al.	35	USA, Alaska	3.0					1960
Cassaret et al.	20	USA, Hawaii	5.7					1968
Kadis et al.	62	CANADA	4.3	1.07*	0.01			1967
Read & McKinley	102	CANADA	4.9					1959
Ritcey et al.	103	CANADA	4.8	0.01*	0.12			1969
Mastromatteo, E.	80	CANADA, Ontario	9.2		0.22			1970

Table 2. Storage of OCC in fat tissue of humans (ppm). South America

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCB	HCB	Year
Wassermann et al.	129	BRAZIL	7.9	0.25*	0.13			1970
Wassermann et al.	126	ARGENTINA	13.2	2.43	0.30			1969
Fernandez et al.	42	ARGENTINA	6.6					1970
Astolfi et al.	10	ARGENTINA	2.7-30.0					1970

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* Only one isomer.

Table 3. Storage of OCC in fat tissue of humans(ppm). Europe.

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCB	HCB	Year
Abott et al.	1	ENGLAND	3.0	0.31	0.21			1966
Abott et al.	2	ENGLAND	2.5	0.29	0.16			1969
Robinson et al.	105	ENGLAND	4.1*	0.01	0.21*			1964
Robinson et al.	104	ENGLAND	4.0*		0.22*			1964
Hunter et al.	58	ENGLAND	2.2*		0.21			1961
Egan et al.	38	ENGLAND	3.3	0.42	0.26			1963
Cassidy et al.	21	ENGLAND	2.6*					1965
Widmark & Jensen	140	SWEDEN	7.3					1967
Bjerk, J.E.	15	NORWAY	3.2			0.85		1972
Weihe, M.	138	DENMARK	3.3	0.20	0.20			1966
Vlieger et al.	120	HOLLAND	2.2		0.17			1968
Maier-Bode, H.	79	GERMANY	2.3					1958
Engst et al.	40	GERMANY, DDR	13.1^{o}					1966
Engst & Knoll	39	GERMANY, DDR				6.40^{o}	5.40^{o}	1971
Wüncher & Acker	142	GERMANY,	4.1	0.56	0.18			1967
Acker & Schulte	4	GERMANY, Muenster reg.	3.8	0.50		5.70	6.30	1971
Borneff, J.	17	GERMANY	5.0					1971
Barchet, R.	11	GERMANY, Neckar	4.9					1972
Hayes et al.	50	FRANCE	5.2					1963
Fournier, E.	44	FRANCE	5.3	0.15				1969
Fournier et al.	45	FRANCE	3.3	0.10	0.40			1971
Jonezyk & Bojanovska	60	POLAND	2-10.0					
Juszkiewisz & Stecy	61	POLAND	12.4	0.13				1971
Ochynski & Bronicz	91	POLAND	16.0					1972
Bogusz, M.	16	POLAND	11.9					1972
Trojanowska et al.	116	POLAND	12.4					1972
Vaskovskaya&Komarova	119	USSR	8.4					1967
Gracheva	47	USSR	0-50.0					1969
Halacka et al.	48	CZECHOSLOVAKIA	9.2					1963
Rosival et al.	106	CZECHOSLOVAKIA	20.3	9.78				1963
Denes, A.	33	HUNGARY	12.4					1960
Denes & Tarjan	34	HUNGARY	24.1					1963
			13.8					1966
Berend et al.	12	HUNGARY	13.1	2.30				1969
Soos et al.	109	HUNGARY	18.9	0.76				1970
Pesendorfer et al.	96	AUSTRIA, Vienna	6.3	1.90	0.10	3.50		1973
Adamović et al.	6	SERBIA	11.5	0.14	0.09			1970
Aizicovici et al.	8	ROUMANIA	21.7					1965
Mandroiu & Jordachescu	78	ROUMANIA, Vrancea		0.08-42.0				1971
Kaloyanova, F.	63	BULGARIA	10.0	0.76				1972
Kanito & Castello	65	ITALY	5.0					1965
Delvechio & Leoni	32	ITALY	15.5		0.68			1966
Pacagnella et al.	95	ITALY	10.8	2.25	0.84			1963
Prati et al.	97	ITALY	9.3		0.07			1966
Llinares & Wassermann	75	SPAIN	14.8					1966
Abott et al.	1	ENGLAND	0.8**	0.14	0.09			1966
Engst et al.	41	GERMANY, DDR	2.4**					1967
Unterman et al.	118	ROUMANIA	4.8**					1970

* Geometric mean

** In stillborn and first days of life

^{o} On a fat basis

Table 4. Storage of OCC in fat tissue of humans (ppm). Asia.

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCBs	HCB	Year
Dale et al.	28	INDIA	12-31	0.86 -1.7	0.03 -0.06			1965
Wassermann et al.	121	ISRAEL	19.2					1963
Wassermann et al.	123	ISRAEL	15.4					1965
Wassermann et al.	135	ISRAEL	14.4	0.47	0.12			1969
Wassermann et al.	133	ISRAEL				2.75		1973
Mughal & Rahman	83	PAKISTAN	25.0					1973
Wassermann et al.	131	THAILAND	13.0					1970
Nishimoto et al.	88	JAPAN, Kochi	6.9	12.2	0.46			1970
Mizutani et al.	81	JAPAN, Kyoto	9.7	11.7	0.19	4.7		1972
Doguchi et al.	27	JAPAN, Tokyo	3.7	3.2	0.33			1972
Curley et al.	23	JAPAN	2.5	1.5	0.13		0.08	1973
Ui, I.	117	JAPAN				5.0		1972
Curley et al.	23	JAPAN				0.8		1973
Kasai, A.	66	JAPAN	8.1	4.3				1972
Suzuki et al.	110	JAPAN, Hiraga	4.5	2.4	0.16			1970
			2.1	3.0	0.21			1971
			4.0	4.0	0.43			1972
Tatsumi et al.	112		4.2	2.4	0.16			1971
Kawanishi et al.	68	JAPAN, Usaga	6.4	2.7	0.13			1973

Table 5. Storage of OCC in fat tissue of humans (ppm). Oceania.

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCBs	HCB	Year
Bick, M.	13	AUSTRALIA	9.5*		0.05			1965
Wassermann et al.	124	AUSTRALIA	9.4	0.68	0.67			1968
Lugg, R.	77	AUSTRALIA	3.1		0.21			1969
Brody & Siyali	19	AUSTRALIA					1.25	1972
Brewer & Grath	18	NEW ZEALAND	5.8		0.27			1966
Darcre, J.C.	26	NEW ZEALAND		0.50				1963
Copplestone et al.	22	NEW ZEALAND	5.4		0.41			1965
			3.9		0.27			1969
Dyment et al.	36	NEW GUINEA				0.0		1971

Table 6. Storage of OCC in fat tissue of humans (ppm). Africa.

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCBs	HCB	Year
Wassermann et al.	127	SOUTH AFRICA	6.38	2.41	0.04			1969
Wassermann et al.	125	NIGERIA	8.75	0.68	0.22			1967
Wassermann et al.	132	NIGERIA	6.50	0.30	0.18			1970
Wassermann et al.	130	KENYA	4.60	0.29	0.10			1970
Wassermann et al.	134	UGANDA	2.90	0.08	0.04			1970

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* Arithmetic mean.

Table 7. OCC residues in mother's whole milk (ppb).

Authors	Ref.	Country	T.DDT	BHC	Diel.	PCBs	HCB	Year
Bjerk, J.E.	15	NORWAY	50-100					1972
Westöö & Norén	139	SWEDEN	113.0					1968
Egan et al.	38	ENGLAND	130.0	13.0	6.0			1965
Knoll & Iayanaman	70	GERMANY	320.0					1971
Acker & Schulte	3	GERMANY	112.0	18.0		103.0	153.0	1971
Engst & Knoll	39	GERMANY	230.0					1970
Kontek et al.	72	POLAND	280.0					1971
Bogusz, M.	16	POLAND	715.6					1972
Gracheva, G.V.	47	USSR	0-1000					1964
Komarova, L.F.	71	USSR, Urban	100.0					1970
Komarova, L.F.	71	USSR, Rural	190.0					1970
Măndroiu&Jordăchescu	78	ROMANIA, Vrancea		0-560.0				1971
Adamovič et al.	5	SERBIA	207.5	5.0				1969
Adamovič et al	7	SERBIA	587.1	14.0	79.0			1972
Laug et al.	74	USA, Black	130.0					1951
Quinby et al.	99	USA	145.0					1965
Curley & Kimbrough	25	USA	70.0	7.0	6.0			1967
Wilson et al.	141	USA, White	170.0					1973
Savage et al.	107	USA	7.495.0	0-38.0	0-11.0	40.0 -100.0		1971
Lofröth, G.	76	CENTRAL AMERICA	3100.0					1971
Olszyna Marzys et al.	92	GUATEMALA	2863.3	0- 100.0				1973
Lugg, R.	77	AUSTRALIA	170.0	2.0	15.0	75.0		1969
Newton & Greene	87	AUSTRALIA	142.0			52.5		1970
Hornabrook et al.	56	NEW GUINEA	29.0-95.9					1972
Gejvall et al.	46	GHANA	29.0	30.0		< 5.0	86.0	1972
Takeshita & Inuyama	111	JAPAN, Agric.	79.0	142.9	0-12.0			1970
Takeshita & Inuyama	111	JAPAN, Nonagr.	66.0	250.9	0-43.0			1970
Kato et al.	67	JAPAN	19-105.0	18- 740	0- 12.0			1971
Oura et al.	94	JAPAN	33.0	49.0		30.0		1972
Anonymous	9	JAPAN	60.2	115.4		3.4		1971
Anomymous	9	JAPAN	56.2	96.0		3.1		1972
Narafu, T.	85	JAPAN		20-400.0				1970
Hidaka et al.	53	JAPAN	95.0	120.0	5.0	50.0		1972
Tattori Res.Hyg.Inst.	115	JAPAN	124.0	109.3	4.7			1972
Kamata, J..	64	JAPAN	90-180.0	70-160				1972
Osaka Pref.	93	JAPAN	21.0	161.5	1.0			1971
Osaka Pref.	93	JAPAN	43.0	180.0	2.0			1972
Hayashi, M.	49	JAPAN, Agric.	56.3	92.6	3.7			1971
Hayashi, M.	49	JAPAN, Nonagr.	63.5	143.4				1971
Nishimoto et al.	90	JAPAN				30.0		1971
Nagai, I.	84	JAPAN, Oshima	20.0	163.0				1972
Nagai, I.	84	JAPAN, Nagato	31.0	219.0				1972
Nagai, I.	84	JAPAN, Yanai	12.0	94.0				1972
Nagai, I.	84	JAPAN, Assa	33.0	247.0				1972

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DISCUSSION

VAN DER KREEK (Netherlands)

I quote your fact 10 "the greatest exposure of the general population, is that of infants fed by mother's milk."

Your showed us a slide with data on concentrations of OCC in fat of infants and neonates. These concentrations in infants do not differ from the concentrations in adults. Therefore I do not understand your fact 10, which is not confirmed by the residues in the fat of the infant. Do you have an explanation?

WASSERMAN (Israel)

At the birth, the OCI storage level in newborns is about 50 percent of their mothers' OCI storage level. There is a continuous increase of the OCI level - a positive age correlation - until the age of about 45 years. In the mother's milk, the OCI concentration is rather high (in Guatemala it was reported at about 3000 ppb) which means a hazardous non-occupational exposure.

OLOFFS (Canada)

1. Considering the difficulties and problems in PCB quantification (number of components and differential metabolism), how valid is it to compare data from the many sources - especially some older ones - and to base conclusions on such data.

2. During a visit to DDR 2 years ago, I had the impression that human tissue burdens of DDT should be rather high in this area as DDT use seemed to be rather prevalent. I find it interesting that the data you presented, Dr. Wasserman, confirm this.

WASSERMAN (Israel)

The LGC method for PCBs assessment is rather more laborious than uncertain. The LGC peaks of PCBs were identified by a number of experienced laboratories.

The present data on stored levels of PCBs in man reported in the literature, which show differences from country to country, may be interpreted in the light of local socio-geographical conjunctures to which the general population is exposed.

REUTER (U.S.A.)

Has any attempt been made to correlate DDT consumption (by nation and years) with tissue levels observed?

WASSERMAN (Israel)

Hayes et al[AMA Arch. Ind. Hlth, 18, 519 (1972)] had found lower levels of stored DDT in vegetarians than in meat eaters, in U.S.A. The same phenomenon was reported by Dale et al[Bull. WHO, 33:471,465] in India. To some extent, a similar situation for Eskimos in Alaska, was also reported by Dale.

HOLL (Federal Republic of Germany)

1. Apart from the fact that the toxicity of DDT is still an open question, and evaluation will have to take into consideration that the Federal Republic of Germany placed a legal ban on the use of DDT on man (Law on the Trade in DDT of 7 August 1972). Exceptions are only applicable to entomology and parasitology.

2. The question of increased concentrations of HCH in mother's milk and cow's milk (vice versa in the infant) was of some importance in the south-western border area of Germany at the beginning of 1974, the reason probably being bound up with waste disposal problems (via soil$<\begin{smallmatrix}\text{air}\\\text{water}\end{smallmatrix}$). Special studies are being carried out and results so far obtained can be made available.

Have you become aware of similar relations? They show the urgency and importance of international arrangements concerning environmental contamination across national frontiers.

WASSERMAN (Israel)

No comment.

EVALUATION OF THE HEALTH EFFECTS OF NITRATES IN WATER

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ABSTRACT

The standard for nitrates in drinking water was established as 45 mg/l based on limited epidemiological and experimental evidence. We initiated a broad range of toxicological and epidemiological studies to provide a scientific basis for evaluating the health effects of nitrates in drinking water. The two epidemiological studies to be reported upon here are part of this project.

One of the studies which was done among 408 healthy infants who consume appreciable amounts of tap water in powdered milk formula showed that those infants who consume water with nitrate concentrations over 45 mg/l exhibited significantly raised methemoglobin levels in their blood. The second epidemiological study was done under controlled conditions in a hospital to attempt to determine the threshold value of nitrates in water which can cause a significant increase above "normal" methemoglobin levels in infants. In this study with 115 infants we showed that nitrate levels in drinking water of about 100 mg/l can cause a significant increase in infants' methemoglobin levels.

The possible health significance of a slightly raised or subclinical methemoglobin level as found in the two studies is discussed in light of results from toxicological studies and general considerations in evaluating the risk of population exposure to chemicals in the environment.

The standard for nitrates in drinking water was initially established based on limited epidemiological evidence that indicated that no cases of infant methemoglobinemia occurred in areas with less than 45 ppm of nitrates (as NO_3) in the water [1]. Since the standard was established there has been considerable controversy on the subject. Some European researchers have reported that they were able to detect raised methemoglobin levels in so called normal infants in areas where occasional clinical cases of the disease were reported [2]. In addition, clinical disease was also reported among infants exposed to water having less than 45 ppm of nitrates. Suggestions that a stricter standard be enforced have been made as a result of such studies.

On the other hand, extensive areas in the U.S. and Europe supplying water showing nitrate levels above the standard have reported little or no clinical cases of the diseases. Since nitrates are difficult to remove from water by economically feasible means moves have been made for a more liberal standard reflecting the lack of clinical disease in such areas.

Since the epidemiological and toxicological base for establishing the nitrate standard was relatively limited, the project reported upon here was initiated to clarify some of the basic questions concerned with the health effects of nitrates in drinking water.

The following general questions served as guidelines in carrying out the evaluation of the suitability of the current nitrate standard in drinking water:

1. Can a dose-response relationship be established between intake of nitrates in drinking water and the development of raised methemoglobin levels in infants?
2. At what threshold level of nitrates in water is the first effect detectable?
3. What environmental, nutritional, physiological or genetic factors influence this relationship?
4. What is the health significance, if any, of chronic subclinical or slightly raised levels of MetHb?
5. Are there direct toxic effects of exposure to nitrates and/or nitrites other than raised MetHb?
6. Can new sensitive parameters be used to detect health effects due to exposure to nitrates other than raised MetHb levels?

In many areas of the coastal plain of Israel concentrations of nitrates in the water supply range from 50-200 mg/l. On completion of the epidemiological study involving 2473 infants in the environs of Rehovot having a mean NO_3 concentration of 70 ppm, it became apparent that there are no significant differences between the mean MetHb levels in the infants in this area as compared to infants of the control area (nitrate level of 5 mg/l [3]. A possible explanation to this lies in the infant feeding practices where only 6% of the infants included in the study received appreciable amounts of tap water together with formula prepared from powdered milk. The Gaza area was considered to be favorable for such a study since preliminary investigations indicated that well over 50% of the infants up to two years of age received powdered milk formula with tap water.

The Gaza area study has provided some confirmation that infants in areas with water supplies having concentrations over 45 mg/l of nitrates who consume appreciable amounts of tap water in powdered milk formula show raised MetHb levels. As can be seen in Fig. 1, infants in the low nitrate group (under 45 mg/l) show similar MetHb levels (mean = .74%) regardless of milk regime while infants who consume powdered milk only in the medium and high nitrates group (46-200 mg/l) have a significantly raised mean MetHb level of 1.18%while those consuming powdered milk and other milk show MetHb of .99%. Although no clinical cases of infant methemoglobinmeia were revealed among 285 infants in the medium and high nitrate group, 26 of them showed significantly raised MetHb levels of over 1.8%; of them 23 (10.7%) received either only powdered milk formula or powdered milk formula in addition to other types of milk. Only 3.2% of the infants in the low nitrate group showed raised MetHb.

Under normal field conditions many difficulties hamper the possibility of detecting a dose-response relationship between nitrate intake and methemoglobin levels especially in the low range. To overcome some of these problems a controlled experiment was carried out in a hospital located in an area normally supplied with water high in nitrate content. In this study we attempted to determine the threshold value of nitrates in water which can cause a significant increase above "normal" MetHb levels in infants.

For five days 104 hospitalized infants ranging from one week to ten months were exposed exclusively to water whose nitrate content was exactly controlled. The infants received mainly formula prepared from milk powder. The exposure schedule was: the first and fifth days, low nitrate content

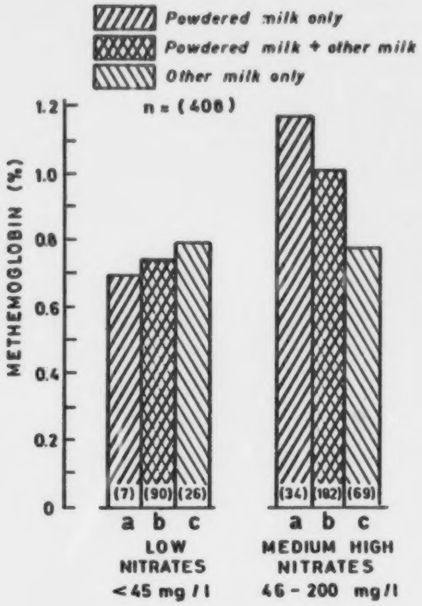

FIGURE 1.

MEAN METHEMOGLOBIN LEVELS IN INFANTS ON DIFFERENT MILK REGIMES, CONSUMING OF LOW AND MEDIUM TO HIGH CONCENTRATIONS OF NITRATES – GAZA.

(15 mg/l), and the second, third and fourth days, high nitrate content (108 mg/l). The high nitrate water was from a well that served as the normal supply to the hospital. There was a significant rise on the second day compared to the first one i.e., following the first exposure to nitrate. The mean MetHb level on the third day decreased almost to the original level in spite of the fact that the high exposure continued. It remained constant on the fourth day (high nitrate intake) but dropped even lower than the first day on the fifth day (low nitrate again). This may hint of an adaptation mechanism.

This work indicates that nitrate levels in drinking water of about 100 mg/l can cause a significant increase in infant MetHb levels. If this exposure is stopped recovery is rapid. It is worthy to note that the mean MetHb level of 22 infants in the Gaza area on "milk powder only" and who received water having high nitrate concentrations (mean 82 mg/l) had a mean MetHb level of 1.37% as compared with a mean of 1.30% for the 104 hospitalized infants exposed to approximately the same level of nitrates in their powdered milk formula.

The Gaza study appears to provide support for the present maximum recommended standard of 45 mg/l of NO_3 in drinking water. The fact that the first signs of raised MetHb levels clearly appear in infants exposed to water just above the standard (45-55 mg/l NO_3) suggests that little if any safety factor is provided by this standard.

The full health significance of slightly raised MetHb levels such as reported upon here is yet to be established. Whether such subclinical methemoglobinemia is deletrious in itself or whether such exposure is only of importance to the extent that clinical cases of the disease develop requires further study.

The question of why and how subclinical methemoglobinemia develops in certain individuals into the clinical form of the disease still remains unanswered. Other possible direct toxic effects of nitrates and nitrites cannot be overlooked as well. Our toxicological studies with rats and mice pointed out the following findings:

1. Nitrites can pass the placenta and cause raised MetHb in the fetus [4].
2. Pregnant rats are particularly sensitive to exposure to nitrites and the pups showed poor growth [5].
3. Rats chronically exposed to sodium nitrate and nitrite in their drinking water for 16 months show distinct deviations in heart blood vessels even at the level of 200 ppm [6].
4. Exposure of mice to nitrites in drinking water causes behavioral effects such as lowered motor activity and an increase in isolation induced aggression [7].

CONCLUSIONS

The results of the two epidemiological studies which encompassed 2891 infants up to 24 months of age indicate that there is a relationship between intake of nitrates in drinking water consumed mainly as powdered milk formula and raised MetHb levels. The effect was detectable and significant even in the group of infants exposed to water containing 45-55 ppm of NO_3. Even though no clinical cases of methemoglobinemia were detected, it is felt that the appearance of a significant increase above the normal MetHb levels in infants when exposed to water with nitrate concentrations slightly above the current standard of 45 ppm is sufficient to provide direct epidemiological support for the current standard. The health significance of such subclinical levels of MetHb is unclear and its is still unknown why only a small number of infants exposed to such levels of nitrates in water develop clinical cases of the disease.

There is still much to be learned about the association of nitrates in drinking water and raised MetHb levels in infants. What role does the powdered milk formula actually play in the development of MetHb? How effective are vitamin rich foods such as citrus juice and tomato juice in preventing raised MetHb? What is their mode of action? Why do only a small percent of infants exposed to high nitrates in water develop clinical cases of methemoglobinemia? A fuller understanding of these questions is still required.

However, despite the many questions that remain unanswered it is apparent that nitrates are potentially more toxic than generally assumed. Until those questions are fully elucidated the standard would best be maintained as is while being kept under constant scrutiny and review.

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RECHERCHES CONCERNANT L'INFLUENCE DE L'ENVIRONNEMENT POLLUE AUX RADIATIONS IONISANTES OU SUBSTANCES CHIMIQUES SUR LES NOYAUX LEUCOCYTAIRES

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RESUME

Les auteurs ont étudié, sur un grand nombre de sujets, les modifications de l'appendice nucléaire au niveau des leucocytes du sang péripherique.

D'une part, les recherches ont porté sur 500 sujets provenant de:
a) malades exposés à des radiations ionisantes (rayons X ou gamma) au cours des procédures thérapeutiques;
b) cadres médicaux et auxiliaires travaillant dans des services de radiothérapie ou la radio-diagnostique;
c) différents sujets venant pour l'examen ou le control périodique médical.

Les analyses cytologiques ont mis en évidence, à un pourcentage varié, mais surtout dans le cas b), la présence de nombreuses excroissances nucléaires sous la forme d'appendices nucléaires.

D'autre part, on a fait des investigations chez les personnes qui travaillent dans un environnement chimiquement pollué (benzène colorants chimiques et alcools), dont on a pratiqué des examens hématologiques. Les analyses ont également mis en évidence les aspects des mêmes modifications cytologiques.

Dans toutes les deux catégories des sujets on a comparé les données en les rapportant à celles des sujets de contrôle, tout en relevant les aspects qualitatifs et quantitatifs de diverses formes d'excroissances nucléaires.

ABSTRACT

The authors have carried out tests on a large number of persons in order to study the changes in the nuclear appendix in peripheral blood leukocytes.

Research was carried out on 500 test subjects taken from the following groups:
a) patients exposed to ionising radiation (x-rays or gamma rays) as part of therapeutic treatment;
b) medical and auxiliary staff working in therapeutic and diagnostic radiology units;
c) various persons coming for examination or periodical medical checks.

The cytological analyses revealed the presence in varying degrees, particularly in the case of group b), of numerous nuclear growths in the form of nuclear appendices.

In addition, blood tests were carried out on persons working in a chemically polluted environment (benzene, chemical dyes and alcohols). Various aspects of the same cytological changes were also revealed by these analyses.

For both categories of test subjects, the findings were compared with those of the control subjects, special attention being given to the qualitative and quantitative aspects of various forms of nuclear growths.

1. INTRODUCTION

Les recherches cytologiques détaillées de Davidson et Smith; Kosenow et Scupin, de même que celles des plusieurs autres chercheurs (Caratzali; Porter; Ruhren; Popovici; De Bernardi et Griva; Asley et Jones; Capra P. Marzani, etc.) sur la fréquence et la morphologie de certaines excroissances nucléaires des leucocytes sanguins ont relevé des aspects particulièrement intéressants d'une grande utilité pour la pratique médicale (2, 3, 4, 7, 17, 18, 19, 21, 23, 25, 28).

Un grand nombre d'autres recherches expérimentales et de laboratoire ont démontré la liaison qu'ont ces appendices nucléaires, non seulement avec le sexe des porteurs (drumsticks), mais également avec l'action nocive de certaines substances chimiques ou de facteurs physiques (mutantes?), qui, se sont montrés susceptibles d'induire, dans certaines conditions, des modifications de la structure nucléaire. (1, 4, 5, 6, 7, 10, 11, 15, 16, 19, 22, 24, 26, 27, 31).

2. HYPOTHESE DE TRAVAIL

Vu les données de la littérature médicale de spécialité quant à l'action nocive de certains facteurs chimiques physiques ou biologiques, sur l'organisme humain en général, et en particulier sur ses structures cellulaires, ainsi que sur la manière du comportament cytologique (microscopique), nous nous sommes proposés d'étudier:

- la fréquence et l'aspect morpho-cytologique des excroissances nucléaires (appendices nucléaires) au niveau des leucocytes;
- les mêmes valeurs, obtenues chez des personnes travaillant dans des milieux pollués par des rayons ionisants ou des substances chimiques nocives, par rapport à un lot témoin.

3. MATERIEL ET METHODE

Le travail a comporté l'étude des 1000 personnes, des deux sexes agées de 18 - 60 ans, qui nous furent envoyées afin d'examiner leur tableau sanguin.

On a fait des recherches sur 3 grands groupes de personnes.

Groupe A, comportant 200 jeunes sujets, sur lesquels on a effectué les examens imposés par leur engagement dans la production.

Groupe B, comportant 500 sujets ayant travaillé effectivement 1 - 20 ans dans des milieux pollués avec diverses substances chimiques telles que: le benzène, les colorants synthétiques, les alcools, l'acétone etc.

Groupe C, comportant 300 personnes divisées en deux sous-groupes: le sous-groupe C-1, comprenant des sujets exposés à l'action des rayons ionisants (rayons gamma, rayons X) à l'occasion de certains procédés thérapeutiques ou bien dans un but diagnostique, et le sous-groupe C-2, dont les sujets étaient représentés par les médecins et des techniciens, qui développaient leur activité quotidienne dans les services de radiodiagnostic et de radiothérapie.

Les frottis de sang périphérique furent colorés par la méthode classique de May-Grünwald-Giemsa et furent ensuite examinés et photographiés au microscope (imersion).

Sans tenir compte de leur signification, nous avons enregistré les types suivants d'excroissances nucléaires (sur 100 neutrophiles examinés chez chaque sujet):

1) Le type "A" (Kosenow et Scupin) dit aussi celui de "l'appendice pédiculé" qui a la forme d'une "baguette de tambour", de "goutte suspendile" ou de "mollet de poule" ("drumsticks", d'après Davidson et Smith). Il s'agit d'une granule chromatique, d'un diamètre de 1,5 microns, bien délimitée et liée au noyau par un mince filament chromatique (fig. 1-a).

2) Le type "B" (Kosenow et Scupin) dit aussi celui des "appendices-sessiles", ou bien "tags" (d'après Davidson et Smith). Ceux-ci apparaissaient comme des granulations chromatiques similaires, comme formes et dimensions à celles de la première catégorie. Elles sont dépourvues de filament d'union, sont sessiles et acollées au noyau (fig. 1-b).

3) Le type "C" (Kosenow et Scupin) ou bien celui des small-clubs" (d'après Davidson et Smith), étant donné qu'elles apparaissaient comme des excroissances nucléaires pédiculées, terminées par une tête en forme de massue, dont les dimensions se trouvent toujours au-dessous d'un micron. Leur aspect est similaire à celui d'une "épingle". (fig. 1-c).

4) Les excroissances nucléaires ayant la forme de certains lobes (Davidson et Smith), sessiles ou plus rarement liées au noyau par 2 ou 3 filaments, moins chromatiques et moins homogènes. Les lobes toujours un diamètre de 2 microns (fig. 1-d).

5) Des excroissances nucléaires de forme conique qui sont des filaments chromatiques ayant une extrémité aigue l'aspect d'une aiguille ou de stalactite, jamais granulées ou lobulées (fig. 1-e).

6) Nous en avons ajouté le groupe des excroissances nucléaires difficilement ou bien impossible à classifier à cause de leurs formes atypiques, non caractéristiques.

4. RESULTATS ET DISCUSSIONS

Nous avons enregistré les résultats de nos observations dans des tableaux et des graphiques, et nous les démontrons par des microphotographies.

De l'analyse des données statistiques offerte par nos tableaux et graphiques, il est a mentionner les suivants aspects plus particuliers:

Le lot témoin (groupe A)

Des excroissances nucléaires apparaissent seulement sur 38,47 % des noyaux leucocytaires surtout pour le type "B" (16,76%). Le type "A" touche des valeurs plus grandes chez le sexe féminin (6,98%) par rapport au sexe masculin (0, 76%). Rapportant les indices de fréquence des divers types d'appendices nucléaires séparément à chaque groupe d'âge, l'on constate une croissance, parallèle à l'âge, des noyaux modifiés, (de 34,40% pour le groupe de 18 - 20 ans jusqu'au 41, 97% pour le groupe de 51 - 60 ans). (tabl. I, graph. nr. 1).

Pour ce qui concerne les personnes travaillant dans un milieu pollué par des substances chimiques nocives, le nombre des cellules dépourvues de modifications nucléaires est diminué d'une manière significative, jusqu'au pourcentage de 40,93%. Le nombre des noyaux comportant diverses excroissances est également élevé, pour les deux sexes, ayant une fréquence presque double par rapport au lot témoin et aux types 2, 3 et 4. Par contre, le nombre des appendices pédiculés diminue, tandis que les formations sessiles augmentent sérieusement et atteignent des valeurs importantes, notamment chez les personnes ayant une activité prolongée dans le milieu pollué (tabl. II, graph. 2, 5).

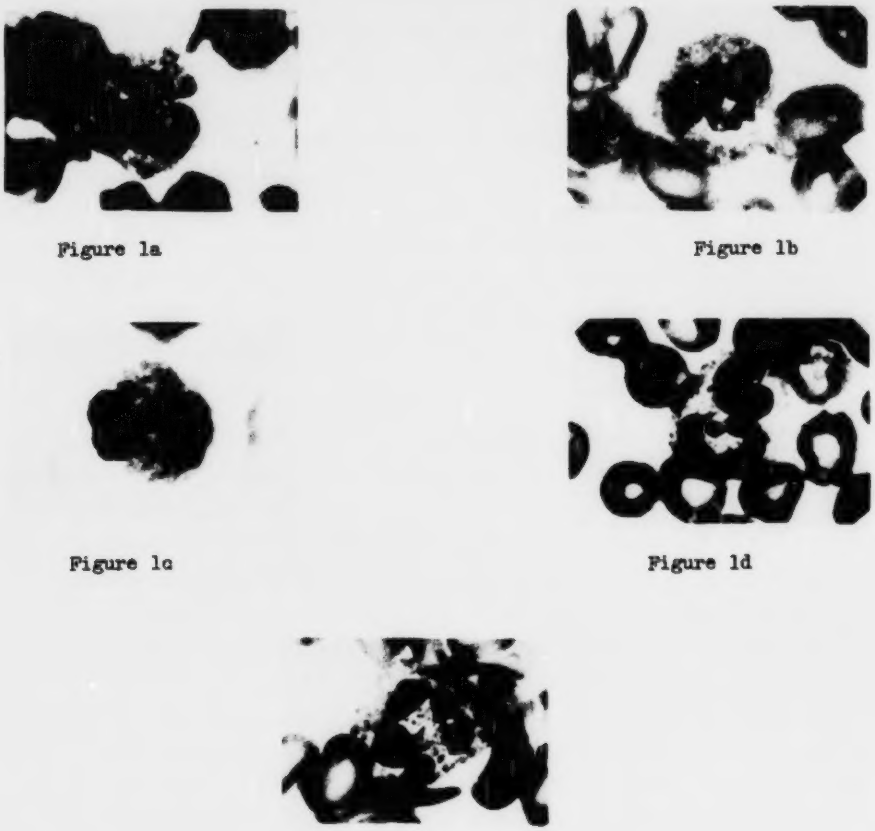


Figure 1a

Figure 1b

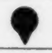
Figure 1c

Figure 1d

Figure 1e

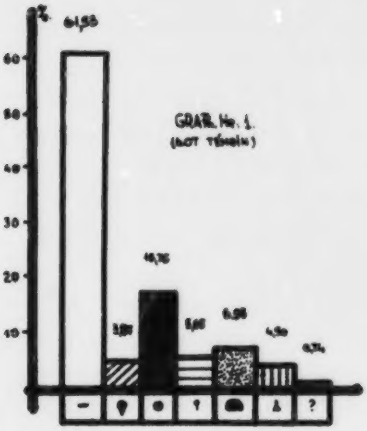
FIGURE 1. Les microphotographies avec l'aspect des divers types d'excroissances nucléaires. (1a "drumsticks"; 1b "tags"; 1c "small-clubs"; 1d "lobes"; 1e "Aiguille")

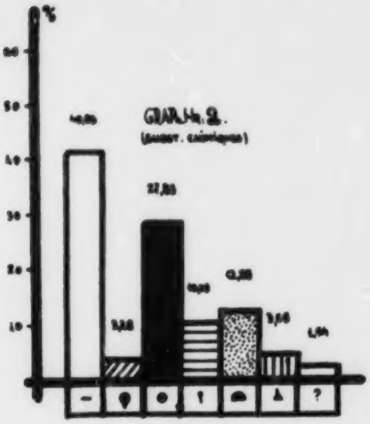
Le tableau n° I
représente les indices de fréquence des divers types d'appendices nucléaires chez les personnes du lot "A" (témoin) séparé en 5 groupes, par âge et sexe.

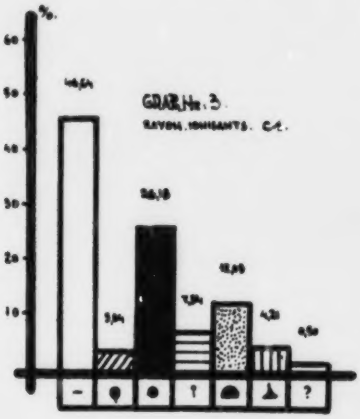
l'âge	Cellules sans appendices			Cellules avec des divers types d'appendices																	
																					
	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total
18-20	66,	65,20	65,60	1,00	6,	3,50	22,	14,60	18,30	1,	5,20	3,10	3,	5,50	4,25	7,	2,50	4,75	0,	1,	0,50
21-30	64,25	60,70	62,48	0,5	8,	4,25	18,	16,80	17,40	3,50	4,	3,75	6,	6,	6,	6,25	3,70	4,97	1,50	0,80	1,15
31-40	59,	63,70	61,35	0,	7,70	3,85	17,	14,	15,50	12,	3,70	7,85	9,	8,30	8,65	3,	1,30	2,15	0,	1,30	0,65
41-50	62,66	57,70	60,18	0,66	7,	3,83	16,50	18,70	17,60	7,	5,60	6,30	5,33	5,80	5,56	7,	4,50	5,75	0,85	0,70	0,78
51-60	55,66	60,40	58,03	1,66	6,20	3,93	16,35	13,60	14,98	7,33	7,22	7,26	12,	8,60	10,30	7,	2,80	4,90	0,	1,20	0,60
MED.G.RA	61,50	61,54	61,53	0,76	6,98	3,87	17,97	15,54	16,76	6,17	5,14	5,65	7,07	6,84	6,95	6,05	2,96	4,50	0,47	1,00	0,74

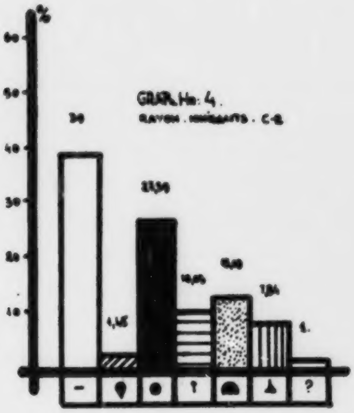
Le tableau n° II
comprend les indices de fréquence des divers types d'excroissances nucléaires pour les 3 groupes des personnes examinées.

Gr.	Cellules sans appendices			Cellules avec des divers types d'appendices																	
				💧			●			⚲			◓			▲			?		
	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total
A	61,50	61,54	51,53	0,76	6,98	3,87	17,97	15,54	16,76	6,17	5,14	5,65	7,07	6,84	6,95	6,05	2,96	4, 0	0,87	1,	0,74
B	38,40	43,45	40,93	1,60	4,95	3,28	27,90	28,47	27,83	12,40	7,78	10,09	14,60	9,97	12,28	4,	3,30	3,65	1,80	2,08	1,94
C-1	50,52	42,76	46,64	1,70	4,56	3,04	20,	32,36	26,18	8,	6,74	7,34	14,	10,18	12,09	6,	2,42	4,21	0,02	0,98	9,50
C-II	42,	56,	39	1,	1,90	1,45	25,	30,18	27,59	5,	15,07	10,03	13,	13,18	13,09	13,	2,67	7,84	1,	1,	1,

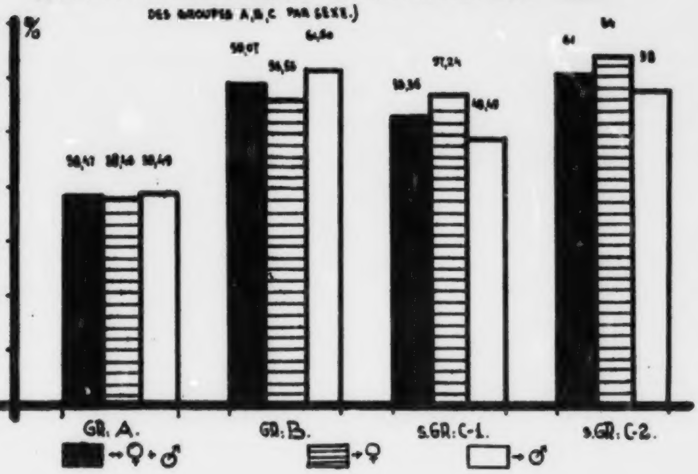








GRAF. No. 5. (LES INDICES DE FRÉQUENCE D'APPENDICES NUCLÉAIRES CHEZ LES PERSONNES DES GROUPES A, B, C PAR SEXE.)



Chez les personnes irradiées pour des fins diagnostiques ou thérapeutiques, les tests cytologiques indiquent une valeur élevée de l'indice général de fréquence des noyaux ayant des modifications de surface (53,36%). Les types 2-3 et 4 d'appendices nucléaires présentent toujours des valeurs presque doubles par rapport au lot témoin. De même l'on a enregistré une diminution des formations pédiculées, même pour celles caractéristiques au sexe féminin (le sexe nucléaire femelle). (tabl. II, graph. 3, 5).

Chez les personnes qui travaillent dans un milieu pollué par des rayonnements ionisants: médecins, personnel technique et cadres moyens auxiliaires (l'âge variant entre 25 - 50 ans), les aspects morphologiques et de fréquence des excroissances nucléaires, mentionnés pour le sous-groupe antérieur C-1, sont encore plus marqués et plus significatifs. A notre avis il s'agit, dans ce groupe, d'une action chronique cumulative du milieu pollué par des rayons sur l'organisme humain. Généralement, voir-même chez les femmes, les excroissances pédiculées diminuent d'une manière manifeste, tandis que les formations sessiles, atteignent, pour ce sous-groupe C-2, les valeurs les plus élevées (tabl. II, graph. 4, 5).

Les données que nous avons obtenues par des analyses morpho-cytologiques et statistiques, effectuées sur un grand nombre de cas, témoignent de la possibilité de déceler certaines modifications de la surface nucléaire. Ces modifications de la surface nucléaire, permettent, outre l'établissement du sexe nucléaire, des estimations sur le degré d'action de plusieurs noxes chimiques ou physiques du milieu pollué sur l'organisme humain en général et notamment sur les cellules leucocytaires. L'augmentation marquée du nombre d'excroissances nucléaires chez les personnes qui travaillent dans un milieu pollué (gr. B-C), se trouve favorisée ou bien constitue l'expression, la manifestation cytologique, de l'action des agents nocifs, sur la surface et le contenu nucléaire. Les modifications peuvent être dues à des mécanismes différents, tel que: l'épaississement de la membrane nucléaire, le plissement de la membrane nucléaire, la pycnose, la karyolyse, la nécrobiose.

En guise de conclusion nous pouvons dire que les tests cytologiques effectués chez un grand nombre de personnes venues en contact avec des milieux pollués, indiquent:

1. L'existence, au niveau des noyaux leucocytaires, de plusieurs types d'excroissances nucléaires, dont certaines reflètent par leur forme et fréquence le degré de nocivité du milieu pollué sur l'organisme et notamment sur les cellules leucocytaires.

2. Ces excroissances nucléaires ayant la forme d'appendices ont une fréquence élevée, et modifient leur forme (en prédominance les formations sessiles) en rapport direct avec l'âge et le temps passé dans le milieu pollué par des substances nocives chimiques ou par des rayons ionisants.

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EXPOSITIONSINDIKATOREN
INDICATORS OF EXPOSURE
INDICATEURS D'EXPOSITION
INDICATORI DI ESPOSIZIONE
INDICATOREN VAN EXPOSIE

(Continued)

Vorsitzender - Chairman - Président - Presidente - Voorzitter

W.J. LLOYD (U.S.A.)

STANDARDIZATION OF ALA-D ACTIVITY DETERMINATIONS AT THE EUROPEAN LEVEL; INTERCALIBRATION AND APPLICATIONS

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ABSTRACT

In view of the difficulties with blood lead analysis, at levels corresponding to a normal environmental exposure to lead, the possibility of using ALAD activity changes for the assessment of such exposures has been explored.

A "European Standardized Method" has been developed, calibrated and tested in an intercomparison programme. The results of these tests compare very favourably both with blood lead and ALA in urine determinations. The interlaboratory coefficient of variation in the ALAD intercomparison programme was of 10%.

The method has been applied within the framework of a small population survey conducted by nineteen laboratories. The preliminary results of this survey seem to indicate that statistically significant differences might exist in ALAD activity levels between similar population groups in different cities.

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Introduction

Lead is amongst the priority pollutants to be dealt with, within the Environmental Action Programme of the European Communities approved by the Council of Ministers on 19 July 1973.

Its universal presence in the environment makes a systematic monitoring of this element in the various media an extremely arduous task.

Attempts have been made to assess man's exposure to lead by measuring the blood lead levels. This approach has led to fruitful results in cases of professional and accidental exposures. The analytical techniques for blood lead determinations at these levels are sufficiently accurate, and most often only likely to be in error by excess, to serve as a diagnostic tool to detect abnormally high exposures to lead.

In order to assess the accuracy and reliability of blood lead measurements at concentrations corresponding to a normal exposure to environmental lead, we have completed two international intercomparison programmes on blood lead measurements designed for this purpose (1, 2). These programmes, carried out at a two year interval, have not shown any marked improvement in the results, in spite of the efforts made by the laboratories in the interval to improve the reliability of the measurements.

It is still possible to conclude that none of the techniques, as currently used by the laboratories, seem to be accurate and precise enough to detect, on one hand the small differences in blood lead levels that might result from different environmental exposures to lead, and, on the other, to compare results obtained by different laboratories.

The discovery that the activity of delta-aminolevulinic acid dehydratase (ALAD) in red blood cells is affected by small changes in blood lead levels, at concentrations normally found in unexposed populations (3, 4) has led us to consider the possibility of using ALAD activity as a bio-analytical tool to assess small changes in environmental exposure to lead.

The recent confirmation (5) that the depression of ALAD activity by lead is an in-vivo effect and not simply an in-vitro artifact strengthens our suggestion that the decrease in the value of the correlation coefficient between blood lead levels and ALAD activities, at low blood lead levels, is mainly due to the increased uncertainty in the accuracy of blood lead determinations.

Chronological development and main features of the "European Standardized Method" for ALAD determination

A small study performed in June 1972 with the collaboration of nine laboratories has shown that ALAD activity ratios can be determined with good reliability, especially in comparison with blood lead or ALA in urine (1), even when each laboratory used its own method.

These results were sufficiently encouraging to set out with the development of a "standard method" having a general acceptability. For comparing results obtained by different laboratories a standard method is needed, since activity determinations are not absolute determinations and since control groups in different areas may have different base lines.

In June 1973 a preliminary common procedure was elaborated by the participating laboratories. From June to September 1973 it was tested by each laboratory with respect to the ease of manipulation, accuracy and reliability. During these tests the sensitivity to light of the porphobilinogen formed was discovered.

In September 1973 at a technical seminar in Erlangen all the participating laboratories had the possibility to test jointly the method, including minor modifications. Some of the results obtained are reported further on. At the end of the seminar the final, currently used, method was approved.

The calibration of the method and some of the results obtained to date in population surveys are described below.

The principle of the method adopted for the determination of delta-aminolevulinic acid dehydratase activity is well known (6).

It is based on the incubation of the enzyme on an excess substrate of delta-aminolevulinic acid. The porphobilinogen formed within a fixed time is mixed with modified Ehrlich's reagent and the colour developed measured photometrically against a blank. The quantity of porphobilinogen produced is a measurement of the delta-aminolevulinic acid dehydratase activity.

A schematic diagram of the procedure adopted is given in Figure 1. The details of the procedure are described elsewhere (6)

SAMPLE PREPARATION	**BLANK PREPARATION**
o,2 ml venous blood + 1,3 ml dist. water	o,2 venous blood + 1,3 dist. water

Hemolysis by 37°C for 10 min.

+ 1 ml ALA-sol.	+ 1 ml TCA-$HgCl_2$-sol. + 1 ml ALA-sol.

Incubation by 37°C for 1 hr.

+ 1 ml TCA-$HgCl_2$-sol.

Centrifugation and Filtration

1 ml of supernatant
+ 1 ml Ehrlich's reagent

5 min. reaction time

Extinction measurement of sampie and blank at 555 nm in 1 cm cell.

Calculation:

$$\frac{\text{O.D. corr.} \times 35 \times 2 \times 100}{\text{Hct } (\%) \times 60 \times 0{,}062} = \mu\text{mols ALA} \cdot \text{min}^{-1} \cdot \text{LRBC}^{-1} = \text{U/L}$$

Figure 1: Diagram of the procedure for the determination of ALAD activity in blood with the "European Standardized Method".

The present method is aimed at assessing activity levels in blood corresponding to low levels of exposure to environmental lead. The steps normally leading to an enhancement of the activity, (i.e. hemolysis with Triton X 100) necessary when determining the ALAD activity of persons exposed to lead, have been omitted.

The method has been designed in such a way that two technicians should carry out the sampling in the field; the number of tasks that have been assigned to be performed during the sampling will reduce considerably the workload to be performed in the laboratory.

For normal blood the optical density values obtained range from 0.5 to 1.0, giving ALAD activities in U/L from 30 to 60.

Calibration of the method and intercomparison study

A first test of the reproducibility of the method was carried out during the technical seminar in Erlangen where the technicians of each of the 16 laboratories analysed a series of 10 identical samples. The average coefficient of variation for these analyses was of only 3% with a standard deviation of 2% (Table 1).

Lab. No.	precision inseries	precision from week to week	precision from day to day, from lab to lab (two blood sampl.)	precision from day to day, from lab to lab, from technicinn to technician (two blood sampics)
	C.V.%	C.V.%	C.V.%	C.V.%
1	2,56	5,43	11,7 or 12,5	9,5 or 13,5
2	2,56	20,30		
3	2,4	21,58		
4	2,8	14,7		
5	7,2	11,26		
6	2,16	8,25		
7	4,1	6,2		
8	0,517	14,01		
9	4,8			
10	1,3	15,22		
11	6,7	7,49		
12	3,45	10,20		
13	---	---		
14	0,6	7,09		
15	0,6´	2,10		
16	---	6,29		
17	---	---		
18	1,73	2,73		
19	4,16	5,00		
$\bar{x}$	2,98	9,87	12,1	11,50
± S.D.	2,02	5,85		

Table 1: Comparison of the variation coefficients for the ALAD determination with the "European Standardized Method".

Following the technical seminar each laboratory was requested to select five persons to act as internal standards. An ALAD activity analysis was to be carried out at least weekly for a minimum of three weeks. A statistical analysis of these week to week results shows a coefficient of variation of 10% with a standard deviation of 6%.

These results were considered sufficiently satisfactory to undertake the next step of the study: an international interlaboratory comparison programme.

The lack of stability in-vitro of ALAD prevented, as in the case of the internal standardization, the simple shipment of blood samples. Two persons, Miss J. Trotter and one of the authors (K.H.S.) volunteered to act as international references and to visit all the participating laboratories. A programme was set up in which in addition to donating blood, they would carry out the ALAD activity determinations alongside the local laboratory and also verify the calibration of the spectrophotometers with standard filters.

The activity values found were as follows:

	Blood Trotter	Blood Schaller
Average as analysed by Trotter	45.9_5	36.0_6
Average as analysed by the local laboratory technicians	46.1_7	36.6_6

The calculated coefficients of variation are presented in Table 1, column 4 for the analyses carried out by Trotter and column 5 for the ones performed by the local laboratory technicians. The slightly larger coefficient of variation obtained by Trotter (12.1 vs 11.5%) can be easily explained by the unfamiliar conditions under which she had to work each time; it can only be concluded that the method, as elaborated, is not sensitive to the technician.

At the same time as determining ALAD activity values on the two international references, a number of laboratories also measured the blood lead values; a coefficient of variation of over 20% was found for these determinations. While already significantly higher than the coefficient of variation calculated for ALAD activities (12%) it is quite acceptable when compared with the interlaboratory coefficients of variation of 43, 52 ar 75% obtained in the intercomparison programme (2).

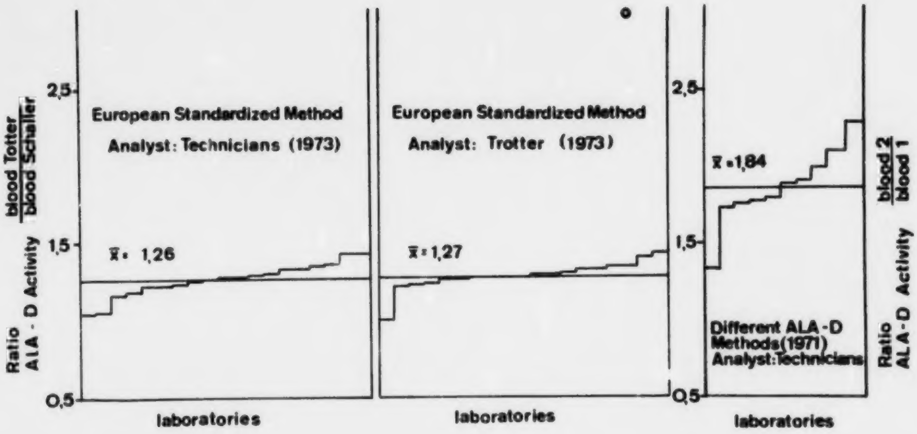

Figure 2: ALAD ratios obtained by different laboratories and Miss Trotter with the "European Standardized Method" (1973) and during an ALAD intercomparison programme (1971) with different ALAD methods.

The comparison, in Figure 2, of the activity ratios obtained by the different laboratories for the two bloods having served as international references using the "European Standardized Method" with the results obtained in the 1972 experiment (1) clearly shows the significant improvement which has been achieved through the standardization.

In Figure 3, a Youden plot of the results obtained for the two blood samples has been made (the 10% tolerance values have been indicated). The plot shows that in some laboratories systematic errors still exist. The possible causes are being examined at present.

The results obtained for the ALAD activity determinations, as expressed by the activity ratios, are compared in Figure 4 with the ratio for blood lead values on the same samples and the results obtained for ALA in urine in the intercomparison programme of 1972 (1). For ALAD, only 15% of the laboratories deviated more than 10% from the average ratio, while more than 50% deviated for both ALA in urine and Pb in blood.

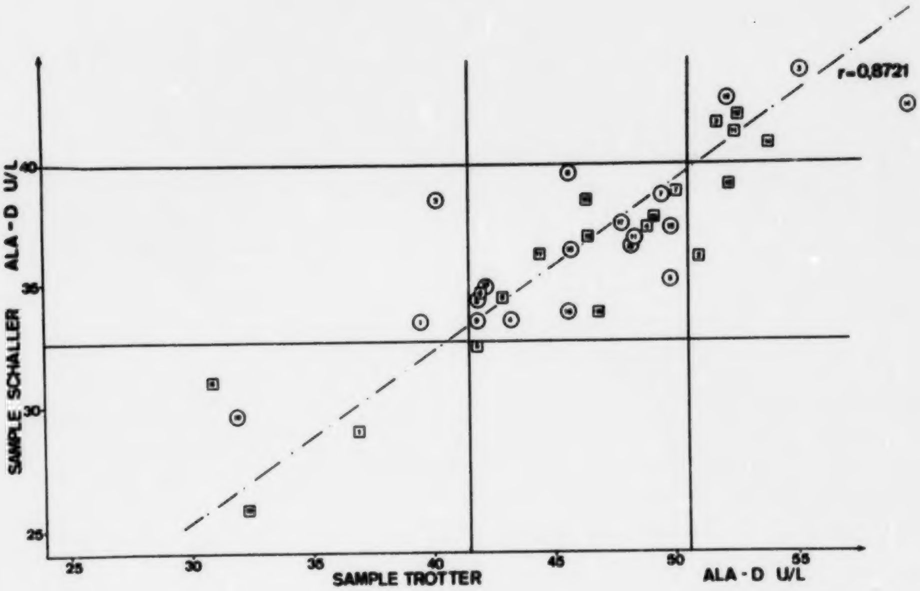

Figure 3: Youden plot of ALAD results from the European intercomparison programme, December 1973/January 1974.

Plot of "high level" sample (Trotter) versus "low level" sample (Schaller). (Magid, E. 1974).

x Laboratory Technician results from lab. no. x.

x Miss Trotter's results.

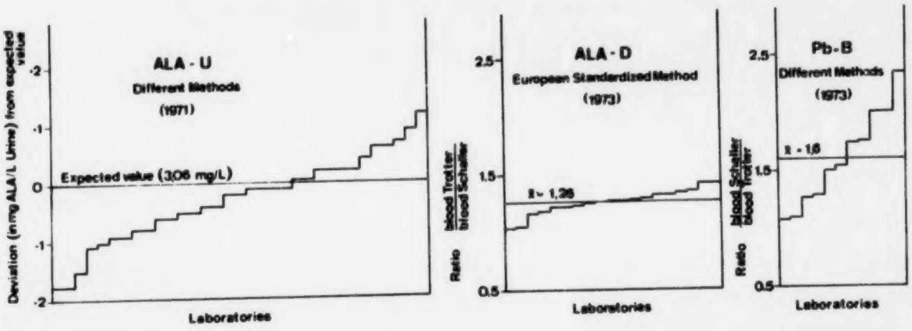

Figure 4: Comparison of ALAU, ALAD and Pb-B determinations.

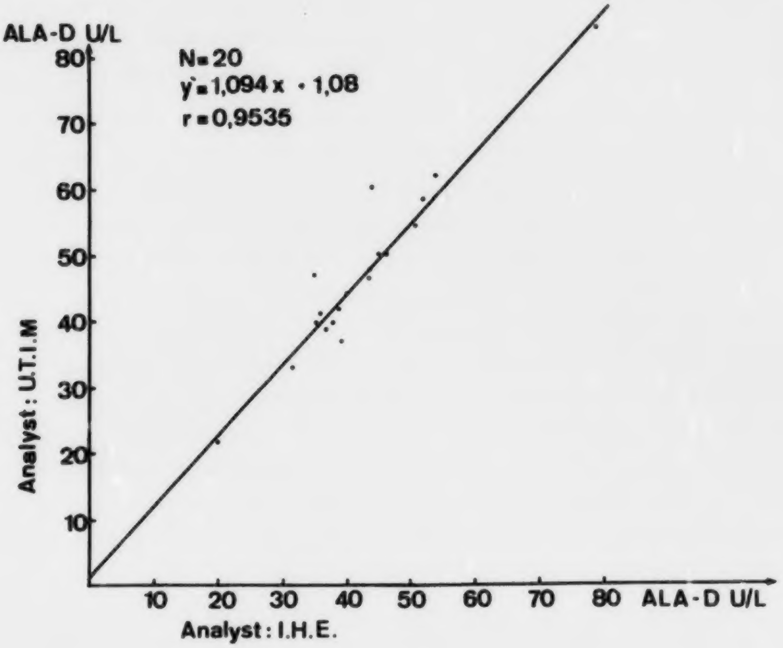

Figure 5: Correlation coefficient and regression line for the relationship between the ALAD results measured by two laboratories in the same city for the same blood samples.

IHE: Institut d'Hygiène et d'Epidémiologie, Bruxelles.

UTIM: Unité de Toxicologie Industrielle et Médicale, Université Catholique de Louvain, Bruxelles.

An additional test of the method was carried out by two laboratories in close geographic proximity to each other; these laboratories carried out a double ALAD analysis on the blood of twenty individuals taken at random. The results are reported in Figure 5. The high correlation coefficient (0.95), the slope close to unity and the small intercept of the regression line, are all indicative of the reliability of the method.

Possible use in population surveys

As indicated earlier, this "European Standardized Method" has been developed as a possible bioanalytical alternative tool to blood lead determinations in assessing small changes in environmental exposure to lead.

We have made a first attempt to use this method to examine if the reported differences in blood lead averages found by various European researchers (i.e. 8, 9, 10) are significant.

Each of the laboratories having participated in the elaboration of the standardized method examined the ALAD blood activity values in groups of 50 males aged between 18 and 40, in principle non-smokers and not engaged in manual work. The groups were so chosen in order to obtain a minimal dispersion of values. Simultaneously, blood samples were sent to "central laboratories" for lead analysis and were also analysed, if possible, locally for this element. While all the ALAD activity analysis results are available and computed, some lead results are still lacking. The results are reported in Table 2. The statistical analysis seems to indicate that the differences are significant in many cases. The correlation with the blood leads already measured was rather poor; it can only be said that the range of ALAD values was smaller than the range of Pb values.

	ALA-D (μmole ALA · min^{-1} · 1 RBC^{-1})		Pb - B (μg/100 ml)	
Laboratories	**Median**	**Range 2,5/97,5 percentiles**	**Median**	**Range 2,5/97,5 percentiles**
Lund (ABDULLA)	49,0	34 - 63	6	4 - 10
Hamburg (ANGERER)				
Genova (BONSIGNORE)	42,9	35 - 51		
Paris (BOUDENE)	32,2	20 - 55	26,4	15 - 44
Bruxelles (BRUAUX)	34,7	18 - 47	19,4	12 - 32
Amsterdam (DE BRUIN)				
Düsseldorf GHELERTER)	29,7	15 - 57	10,2	9 - 12
Eire (GRIMES)	41,2	29 - 65	12,0	7 - 30
Lausanne (GUILLEMIN)	44,9	28 - 61		
Helsinki (HERNBERG)	41,2	28 - 55	8,2	4 - 22
Luxembourg (HOFFMANN)	34,6	23 - 57		
Bruxelles (LAUWERYS)	38,6	23 - 57	23,3	9 - 39
Köbenhaven (MAGID)	45,0	33 - 65	6,8	2 - 26
Glasgow (MOORE)	47,3	25 - 69	22,7	16 - 35
Zürich (ROSENMUND)	40,1	25 - 56		
London (SAYERS)	29,8	19 - 43	18,3	10 - 33
Erlangen (SCHALLER)	31,5	21 - 46	15,9	10 - 24
Milano (SECCHI)	27,3	3,0- 43		
Berlin (WAGNER)	40,3	34 - 49		

Table 2: Results for ALAD and Pb-B by population studies on identical groups in different countries.

Measurement of ALAD activity in human blood samples using the "European Standardized Method".

Conclusion

One must await the complete blood lead results before definite conclusions may be drawn from this population survey, but already the narrow range of values obtained in a homogeneous population indicates that the biological variability in ALAD activities for a given population is not larger than the variability (biological and/or analytical) in blood lead values (11).

The reproducibility and precision of the method, its low cost per analysis, the easy implementation by many laboratories (no elaborated equipment required) should allow this method to be used as a possible alternative to blood lead determination when carrying out population surveys of exposure to lead or when assessing a local situation with respect to a set of "biological quality guides" (12).

Acknowledgements

In listing the laboratories having participated in this programme the authors wish to acknowledge the fact that the elaboration of the "European Standardized Method" has only been possible through their efforts.

The authors wish to thank Madame Langevin and Miss Trotter for their technical contribution and for the coordination of this programme. Special thanks are also due to Professors Recht and Valentin for their advice and encouragement during this whole study.

List of participating laboratories

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13. Universiteit van Amsterdam, Nederland (A. de BRUIN)
14. University of Glasgow, Department of Materia Medica, UK (M. R. MOORE)
15. Department of Employment, Central Reference Laboratory, London, UK (M. H. P. SAYERS)
16. Department of Epidemiology and Biometry, Institute of Occupational Health, Helsinki, Finland (S. HERNBERG)
17. Forskningslaboratory, Lund, Sverige (M. ABDULLA)
18. Institut Universitaire de Médecine Sociale et Préventive, Lausanne, Suisse (M. GUILLEMIN)
19. Kantonspital Zürich, Schweiz (H. ROSENMUND)

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DISCUSSION

ZIELHUIS (Netherlands)

The speaker suggests ALAD measurements as a possible alternative for Pb in blood levels in monitoring Pb exposure for population groups. However, in population surveys the investigator is often limited in his degrees of freedom to organize the taking of blood; he has to make use of the opportunities given to him by circumstances, e.g. one may have to take blood at night, on Friday. Blood samples have to be investigated within about 2 hours for measurement of ALAD, but they can be left in the laboratory for some days in the case of measurement of Pb. Therefore, ALAD measurement for monitoring of population groups will meet many more logistic problems than Pb in blood measurement.

BERLIN (C.E.C.)

We are aware of these logistic limitations, which are not quite so severe since up to 5 hours can elapse between sampling and analysis. Methods for increasing the conservation time are still being investigated.

However, in view of the quality of the results obtained at present for ALAD in comparison with blood lead, we feel that the increased logistic difficulties are warranted.

LEAD SURVEY OF CHILDREN - ARGENTON - BOOLAROO NEWCASTLE - AUSTRALIA

K. H. OUW AND A. BELL

Division of Occupational Health and Pollution Control - Health Commission of New South Wales, Australia

(paper presented by S.R. Leeder, U.K.)

ABSTRACT

A survey of possible health effects due to contamination of the environment by lead emissions from the Sulphide Corporation Pty. Ltd., Cockle Creek, New South Wales began in 1972 and was continued by the Medical Branch of the Division of Occupational Health and Pollution Control in 1973.

While the results in 1972 did not indicate that the children in the Argenton-Boolaroo area were at any risk from abnormal lead absorption, the survey at that time was not considered by the Division to be adequate, as it was based predominantly upon lead-in-urine results (n = 257) and only to a lesser extent upon lead-in-blood results (n = 41).

At the International Symposium on Lead in Amsterdam in 1972 the matter of environmental lead pollution was discussed, and it was decided to adopt the recommendations set down by R. Zielhuis in his paper "Lead Absorption and Public Health, an Appraisal of Hazards" (as amended), that is, that 50% of the children tested should have a lead level of 20 µg or less per 100 ml. of blood, 98% should have 35 µg or less per 100 ml. of blood, and 100% should have 40 µg or less per 100 ml. of blood, as adequate indications that no health hazard due to lead absorption exists. Accordingly, a further 204 children and infants were investigated,

and blood lead levels determined. Haemoglobin levels were also checked.

The haemoglobin levels were found to be normal for age in all children and infants tested, and results of the 204 lead-in-blood tests showed that 100% had 40 μg or less per 100 ml. of blood, 98% had 35 μg or less per 100 ml. of blood and 83% had 20 μg or less per 100 ml. of blood.

As a result of all the tests carried out in 1972 and 1973, no public health hazard due to abnormal absorption of lead could be demonstrated in the vicinity of the Sulphide Corporation lead smelter at Cockle Creek.

1. Introduction

Excessive lead absorption in children due to the ingestion of lead-containing materials, such as paint flakes, has been widely reported in the literature (Gibson, 1892; Henderson, 1954; Freeman, 1969). Excessive absorption due to lead in ambient air, and residence in areas close to emission sources of lead, is less well documented, and still a matter of contention. This survey reports on the lead absorption of children residing close to a large lead-smelting complex (Sulphide Corporation Pty. Ltd.,) known to be an emission source of airborne lead. 770 workers are employed by the Corporation, and current annual capacity for the chief products is of the following order:

Zinc metal	70.000 tons
Lead bullion	28.000 tons
100% sulphuric acid	130.000 tons

The smelter is located at Cockle Creek near Newcastle, New South Wales, Australia, and the townships of Argenton and Boolaroo are situated North and South-West of the lead smelter respectively. The residents of these townships living within a radius of 2 km of the lead smelter, might reasonably suspect that their health could be affected by absorption of lead from the environment. The route of such absorption might be by inhalation of airborne lead, or by ingestion of contamined food. Children and infants might be especially at risk, due to their predilection for absorbing environmental lead.

A lead survey into possible effects was carried out in 1972. The results did not indicate abnormal lead absorption. However, the 1972 survey was not considered to be adequate, as finding were based predominantly upon lead-in-urine results (n = 257) and only to a lesser extent upon lead-in-blood results (n = 41).

The lead-in-blood level is now regarded as the sole valid criterion (Steinfeld 1971).

A further survey, based entirely upon lead-in-blood tests and haemoglobin values, was therefore conducted in 1973.

2. Method

Two hundred and four children and infants were included in the survey. They were drawn from both Argenton and Boolaroo, in response to an appeal to parents to submit their children for testing. The blood lead and haemoglobin levels for each child were estimated. 50 /ul quantities of whole blood, standards and secondary controls were sampled by means of an Oxford Sampler, and placed in a Spinko microcentrifuge tube. Red cell lysis and chelation were performed using Saponin-Ammonium Pyrrolidine Dithiocarbamate reagent. After mixing in a vortex mixer the chelated lead was extracted into methyl isobutyl ketone and the extract tested in a Varian Model 61 Carbon Rod Atomizer. The unknowns were read against the standard calibration curve. By questionnaire, information from parents was sought to determine whether the child indulged in "pica", or was the child of a lead-smelter worker, and the geographical location of each child's residence.

Levels of lead in ambient air of the residential areas were obtained by the Air Pollution Control Branch of the Division of Occupational Health and Pollution Control.

3. Results :

Histogram 1.

Shows the distribution of the blood lead levels in the 204 children. As can be seen from the histogram, the frequency distribution of the blood lead levels was UNIMODAL, with a mean of 15 µgm/100 ml. of blood, and a standard deviation of 5.94 µgm/100 ml.

Histogram 2.

Shows the age distribution of the children in the survey. The majority were school children in the age range of seven to twelve years old. Twenty one were less than three years old. The youngest was a baby aged ten months; the oldest child in the survey was fourteen years. There was no significant correlation between blood lead levels and age (correlation coefficient = 0.19).

Histogram 3.

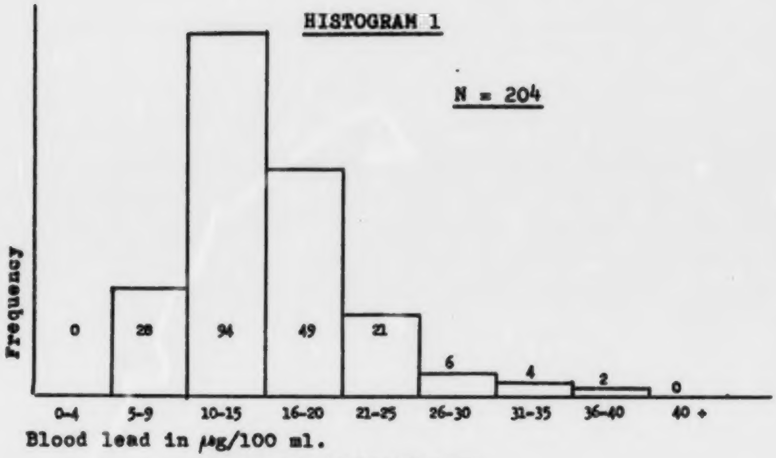
Shows the distribution of the haemoglobin estimations on the children. No child had a haemoglobin level below 10 g per 100 ml. and none of the children were considered to be anaemic.

Tables I - V.

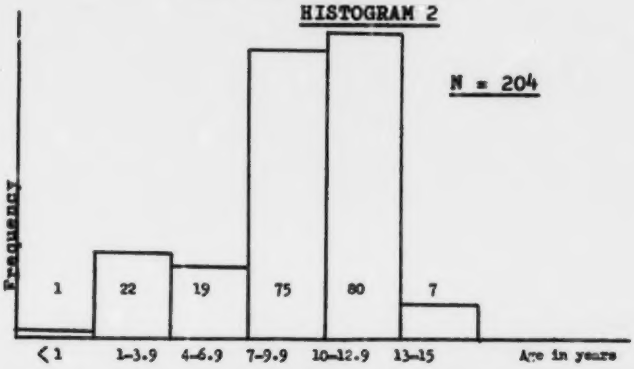
Show the blood lead results for certain sub-groups from the total survey population, who were possibly at greater risk of lead absorption, due to their social, or geographical circumstances.

AIR POLLUTION DATA

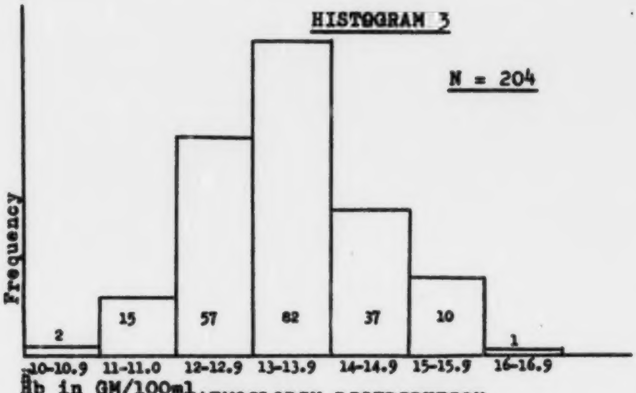
Ambient lead in air: Measurement of ambient lead in air, in the vicinity of the lead smelting complex at Cockle Creek was commenced on the 27 June 1972. Initial measurements (up to 21 July 1972) from 30 sample



BLOOD LEAD DISTRIBUTION.



AGE DISTRIBUTION OF CHILDREN EXAMINED



HAEMOGLOBIN DISTRIBUTION

TABLE 1 Blood lead and Hb. levels of children whose parents work at Sulphide Corporation Pty. Ltd.,

N = 7

Child Identification No.	Blood Lead µg/100ml.	Hb. gm/100ml.
39	13	13.6
113	12	12.3
123	14	12.4
124	7	12.4
150	17	15.4
177	9	14.3
159	19	15.4
Mean	13	13.5

TABLE II

Occupation of parents	No. of Children	Average Blood Lead µg/100ml.	Standard Deviation
In Sulphide Corporation	7	13.0	4.3
Not in Sulphide Corporation	147	14.91	5.47

TABLE III Blood lead and Hb. levels in children with definite History of "PICA"

N = 17

Mean = 15 μg/100ml.

Child identification No.	Blood Lead μg/100ml.	Hb. gm/100ml.
53	23	11.3
68	15	12.8
80	25	12.8
84	14	14.7
85	10	13.7
86	16	13.7
90	25	12.5
91	28	13.6
92	29	13.7
186	17	12.6
187	11	11.6
189	14	12.3
205	14	12.4
206	21	12.2
207	18	12.2
44	11	12.8
17	19	12.8

TABLE IV

History of "Pica"	No. of children	Mean blood lead μg/100ml.	Standard Deviation
Yes	18	18.2	5.8
No	125	14.2	4.9

TABLE V

Place of Residence	No. of Children	Mean Blood level - μg/100ml.	Standard Deviation
Argenton	93	15.5	5.8
Boolaroo	77	14.9	5.8

sites at various distances ranging from 275 to 2000 metres from the works gave an average value of 9.6 μgm/m^3 (range from 0.15 - 31.6 μgm/m^3, 24 hour period samples). Lead emission control at the works has been improved since 1972. From the beginning of August 1972, 5 fixed monitoring stations were chosen, and sampling was carried out on a rotational basis in accordance with the E.P.A. Standard procedure. (E.P.I. Federal Register). The average lead in air from August 1972 was 2.2 μgm/m^3.

4. Discussion

At the International Symposium on Lead in Amsterdam in 1972 the matter of environmental lead pollution was discussed, and it was decided to adopt the recommendations set down by R. Zielhuis in his paper "Lead Absorption and Public Health, an Appraisal of Hazards" (as amended, Zielhuis 1972) that is, that 50% of the children tested should have a lead level of 20 μg /100 ml. of blood, 98% should have 35 μg or less per 100ml of blood, and 100% should have 40 μg or less per 100ml. of blood, as adequate indications that no health hazard due to lead absorbtion exists. The lead-in-blood level is now regarded as the sole valid criterion. Using this corrected "Amsterdam" permissible percentile distribution standard, the distributions in this survey among the children were: 83% of children had blood lead levels of 20 μg/100ml. or less, 98% had levels of 35 μg/100ml. or less and 100%, 40 μg/100ml. or less. Thus, it can be seen that the distribution for lead in blood in this survey is within normal limits.

The distribution of haemoglobin levels among the children surveyed are shown in Histogram 3. For our survey population the normal level of haemoglobin varies from 11.8 gm/100ml. for the child of 10 months to 13.4 gm/100ml. for the child of 14 years. The generally agreed definition in New South Wales of the lower limit of haemoglobin below which anaemia is considered to be present ranges from 10 gm/100ml. at birth rising to 11.0 gm/100ml. at 14 years. (Hendry 1973, Lovric 1973 - Personal communications). From Histogram 3 it can be seen that none of the children in this survey could be regarded as anaemic.

A lead survey in Bristol, U.K., (Report of a Committee under the Chairmanship of Sir Brian Windeyer appointed to inquire into Lead Poisonings at the RTZ Smelter at Avonmouth) showed that there was an increased risk of lead absorption among lead smelters children because of lead dust brought into the home by their fathers. Tables 1 and 11 show blood lead and haemoglobin levels of seven children in our survey whose parents work at the Sulphide Corporation Pty. Ltd. Analysis shows that there was no significant difference in the mean blood lead levels between the group of Sulphide workers children and the children whose parents work elsewhere ($p > 0.05$). This finding is not surprising, as lead workers in the Sulphide Corporation don clean work clothes before they start their shift, and change back into street clothes at the end of their shift. There is thus no reason to suspect that these workers bring lead dust into their home environment.

Pica

Children exhibiting the pica habit are also more at risk from lead poisoning than children without this habit. "Pica" is defined as eating unnatural foods, including dirt, plaster and paint flakes. In the first year of life, it takes the form of mouthing, but not ingestion, of any object the infant can grasp. Later on, the child begins to eat non-food substances, but the habit usually disappears when the child is between 3 and 5 years of age. Emotionally disturbed children tend to acquire this habit. (Chisholm, Jr. 1970). In this survey, pica was considered present in children of any age when the answer to the following question on the questionnaire form was in the affirmative. "Has your child ever had the habit of chewing or eating any non-food items such as toys, old paint, soil etc". Children with a history of "pica" were compared with those without a history of "pica". The total of respondents was 143, 18 of whom gave a history of "pica". (Tables III and IV.).

No statistically significant difference in the mean blood lead levels was found. ($p > 0.05$).

As far as this survey is concerned, children with a history of "pica" did not show an increase in lead absorption. Children, of course, often place non-food items in their mouths, and some mothers may have given negative answers to the "pica" question for fear of being told that they had not trained their children correctly.

Place of residence

Comparison of possible differences in lead absorption by children living in the townships of Argenton and Boolaroo was also carried out. Argenton is north of Sulphide Corporation and Boolaroo is south-west of the industry, and both areas are approximately the same distance from the Sulphide Corporation. The Standard Wind Analysis in the area (combined summary 9099 and 1500 Hr. reading, 1967-1971), showed that the prevailing wind drift from the Sulphide works was towards Argenton. Figures available for 1972 and 1973 show the same trend. Assuming other factors remain equal, and that lead pollution from the Sulphide works is an important factor in lead absorption among children, then we would expect a greater degree of absorption in children living in Argenton than in Boolaroo. Table V shows the mean blood levels of the Argenton and Boolaroo children. No significant difference was found between the blood lead levels in the two groups. ($p > 0.05$).

In summary, judged by the internationally accepted criteria (Zielhuis, 1972), none of the children surveyed showed an abnormally high blood lead level, or low Hb. level, and none of the potentially high exposure groups had elevated blood lead levels. The children surveyed were not a random sample, but they did comprise about 50% of all school-age children in the area, and we have no reason to think that they were unrepresentative of all children in the area. Comparison with other studies of blood lead levels must be made with caution. Differences in socio-economic status, age, and perhaps most importantly, in laboratory analysis can all hinder a true comparison between sample groups. However, the results of this survey do support the initial conclusions of an earlier survey done at Cockle Creek in 1972: there was no excessive absorption of lead among children examined, despite their proximity to a lead smelting complex.

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DISCUSSION

HARRISON (U.K.)

Was the particle size distribution of the airborne lead measured? This would be highly relevant to pulmonary retention.

LEEDER (U.K.)

The lead particle size was not determined in that survey, but in 1972, the Division of Occupational Health and Radiation Control did make a study of the lead particle size, and the conclusion was:

> "the percentage by weight of particles smaller than 5 microns varied between 74 and 39 percents of the total with an average of 55%".

RECENT EPIDEMIOLOGICAL STUDIES OF ENVIRONMENTAL LEAD OF INDUSTRIAL ORIGIN

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ABSTRACT

During the past three years Local Authorities in the United Kingdom have carried out a number of epidemiological surveys in the vicinity of lead works. In some of these the proportion of children and sometimes of their mothers with raised blood levels living in the vicinity of the works was higher than expected. The levels were highest in those living close to the works. Further epidemiological studies showed also that the families of lead workers, even when living some considerable distance from the works sometimes had markedly raised blood levels. In the latter, the source of the raised lead intake was believed to be lead particles on the person or clothing of the workers, notwithstanding the provision of changing and shower rooms at the works. In the case of families living in the immediate vicinity of the works the respective importance of the pathways by which lead was leaving the works could not be ascertained. High lead concentrations were found in the dust within and in the vicinity of the houses and this was thought to be due in part to dust leaving the works on the footwear of employees, on vehicles and particularly on the wheels of vehicles. Some airborne or deposited lead derived from ventilation flues and chimneys would also be present. In one case where particularly heavy pollution was obviously coming from the works the factory management voluntarily agreed to the closure of the plant pending the installation of more satisfactory filtration equipment.

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Individuals with seriously raised blood levels were investigated further and as far as possible other sources of excessive lead ingestion were excluded. In no case was any individual found with clinical symptoms of lead intoxication. The attention of the Medical Officers of Health throughout the country was drawn to the importance of investigating lead pollution from all sources. The Departments of Employment and of the Environment took immediate action to tighten pollution control and a Code of Practice produced by the former, has now been issued laying down methods by which pollution of industrial origin may be minimized. The surveys indicate the need for periodic checks to ensure the effectiveness of the anti-pollution measures adopted by industry.

Introduction

Towards the end of 1971 the London Borough of Tower Hamlets reported strong evidence of lead pollution in the neighbourhood of a large lead works. This particular area is in the middle of London and therefore an urban site and the contamination arose from a smelter from which the lead was being emitted from the chimney. The pollution problem was therefore mainly in the vicinity.

The second site which we wish to discuss is derived from an extremely large smelter in an area which is an industrial estate surrounded by countryside. The contamination is from several sources with high level emission and low level emission with some take home problems, the cattle and vegetation in the neighbourhood being involved to lesser extent.

The third area is semi-rural with a lead mill and chemical factory as the sources. This was not such a severe problem as the two previously mentioned but has certain particular facets of interest.

The fourth episode derived from old lead mines which exist in the area on which a smelter has now been built. This is essentially a rural neighbourhood and the problems which existed were derived partly from take home lead and partly from the high lead levels in the soil from the proximity of the mines.

The fifth site was again a London based source, the neighbourhood being mainly docks with many industries in the area. There were houses around which were mainly blocks of flats built before the turn of the century, some of these being very close to the factory area. The emissions came from a high level from the chimney and the problems were essentially vicinity ones.

Physical Data

In surveys of this sort undertaken by a variety of different people the data is not always as homogeneous as one would wish. We have in most cases managed to get reasonably complete information. The first area in our discussion has houses close around the factory the nearest being approximately 100 meters away and we have examined in the survey the houses up to 500 meters. Being an urban site, there is little soil which is available for analysis but dust has been examined in the gutters and other appropriate sampling places, and the figures obtained ranged up to 1300ppm of lead. Dust was also gathered from the rooves in the neighbourhood and some samples were up to 5,000ppm of lead. Lead in air samples gave a mean daily concentration of $3\mu g/m^3$ with a maximum 24 hour figure of $28\mu g/m^3$. Grass and vegetation were examined, and the figures for grass were of the region of 500ppm lead while plane tree leaves were found to have 1800ppm lead.

At the second site the nearest house was 2km from the smelter and therefore from the environmental point of view the house contamination problem was likely to be of relatively small importance. Lead in air samples taken in the works canteen gave average values from 1.1 to 7.9 $\mu g/m^3$. Heavy deposits of lead were found on vegetation in the vicinity. The outer leaves of green vegetables within one mile of the smelter yielded 32ppm, from one to one and one half miles 16ppm and at a distance of one and one half to two miles 8ppm.

The third site had houses 75-250 yards from the works. Dust at 150 yards away ranged from 10,000 to 50,000ppm lead, and soil samples in the gardens of these houses all contained less than 3,000ppm.

In the fourth situation as one would expect the soil had very high lead values 2,000 to 45,000ppm and the lime stone dust content in the neighbourhood ranged from 175-270ppm. Grass in this neighbourhood had 15ppm of lead. Dust in the area ranged from 300-32,000ppm lead when obtained from the children's houses.

The last site in London had a very high dust level in the vicinity of the factory; the levels of lead ranged from 2,000-80,000ppm; and mud since soil is not readily obtainable in this neighbourhood ranged from 1,000-19,000ppm.

Blood Lead Values

At the first works which had a vicinity problem, pre-school children were examined, that is children under 5 years. 16 of 39 children were found to have blood lead values of greater than the maximum acceptable level of findings represent a 40 % increase in blood lead of those children who lived between 100 and 400 meters from the factory. These findings compared with pre-school children living 400 to 500 meters from the factory where 11 out of 80 had blood lead values which were raised, that is 13.7%, and only one was over 60µg/100ml. Of the mothers who were examined, those living between 100 and 400 meters from the factory 3 out of 25, that is 12%, had raised blood lead values but in the group which lived 400-500 meters from the factory there were none out of the 53 examined whose blood lead findings were increased.

In the second survey which did not have a vicinity problem the nearest houses were 1,600 meters from the factory and in consequence there were no raised blood lead findings in those children that were examined. In the other London borough where the children were examined, those who lived in houses less than 500 yards from the factory 124 out of 220 children had raised blood findings, of these 8 were greater than 60µg/100ml.

Workers families have been found also to be at risk. In the first survey the workers children were not separated from the other children but on subsequent examination of the data it was observed that the only 2 workers' children who were sampled had higher values than their colleagues. In the second study it was noted that the blood lead findings of the children expressed on a frequency distribution curve showed a shift to the right. There were actually only a small number in this study who had blood lead levels of greater than 40µg/100ml. In the third study the control group children were found to have rather high levels, the reason for which is not known. 10 out of 93 had blood lead levels in excess of 40µg/100ml, whereas of the workers' children 7 out of 13 were also high. This difference must be important. In the fourth study 13 out of 31 children had blood lead values greater than 40µg/100ml. These children had a mean value for their group of 43µg/100ml, while control groups which were available had mean values of 22 and 25µg/100ml, respectively so that the difference between the control values and the exposed group is a significant one.

Consideration of the Results

Blood lead values are difficult to interpret in that there are problems with sampling and with the technical analysis. If the sampling is to be done on children and particularly where a repeat sample may be necessary it is desirable to use a capillary sample rather than undertaking venipuncture. On the other hand capillary samples are much more likely to be contaminated and therefore have a higher failure rate and a more frequent need for a second sampling than using blood from a venipuncture. It should however be noted that where skin cleaning is undertaken prior to obtaining a capillary sample the skin swabbing must be done with care and must not be skimped otherwise spuriously high results may be obtained. It is also important that the blood is free flowing and should not be squeezed out of the ear or fingertip. The technical variations have been noted in an inter-laboratory study which is being carried on in the U.K. The results of this are not yet available but the conclusion which appears to be a likely outcome is that where blood lead estimations are being done in large numbers by a single person using one technique, the estimates are more likely to be consistent and reliable. Where only a few are being carried out there is less likelihood of the consistency and reliability which are needed for such measurements.

It has been our custom not to accept a single raised blood lead as being representative of the individual's lead profile. In each case a repeat sample has been examined usually obtained at a clinic or hospital rather than in the atmosphere of the home which may have a high lead in air characteristic.

Subsequent Action

Where the repeated blood lead value has been found to be raised the individual has been referred for a further medical opinion. In each case the general practitioner was informed and where appropriate the paediatrician was asked to handle the subsequent treatment and progress of the child. In no case has any child or adult been found to have any deleterious effects which could be attributed to the excessive ingestion of lead.

The next point with regard to subsequent action is that of considering from where the lead has come. It is important to exclude other sources of lead such as paint from woodwork or off toys, eye make-up and so on, and having excluded all individual sources the environmental situation for the affected people as a whole should be considered.

Other Investigations

There are still many aspects of the lead problems which are not fully understood;for example there is still discussion as to how far excessive lead intake in a child can be related to hyperactivity or to an alteration in behaviour. As a result of the first investigation which we have been discussing an additional study was undertaken into the intelligence, reading ability and behaviour of the group of school children. The Weschler Intelligence Scales were used, and using school children it was concluded that the lower levels of intelligence and higher rates of behavioural disorder were found to be related to social factors rather than the effects of lead.

Conclusion

Each study had its own characteristics in the form of the different type of lead source and the geography of the neighbourhood. We have used the blood lead values as our criterion of excessive lead ingestion, but we would like to have other tests which would indicate early toxic effects. The number of blood samples was small in relation to the number of people exposed so that the techniques used provided relatively coarse assessments of the situation.

Acknowledgments: We must express our thanks to all who took part in the organization and conduct of these surveys and in particular to Professor Barbara Clayton, Dr W C Turner, Mr P Broughton and Dr D Barltrop, to the Medical Officers of Health responsible for the local organization of the surveys in their areas (Dr R Adams, Darley Dale; Dr G K Brown, Greenwich; Dr E M Hargreaves, Rothwell; Dr R Kind, Market Harborough; Dr J E Epsom, Southwark; Dr W Maughan, Beverley; Dr P F Morgan, Chester; Dr. R G Taylor, Welwyn Garden City; Dr R W Watton, Tower Hamlets; and Dr R C Woffinden, Bristol) to the various laboratories and to the factory managements who so willingly cooperated.

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DISCUSSION

EPSTEIN (U.S.A.)

The results of the reported studies, like previous studies in children living in vicinity to smelting plants in El Paso, Toronto, and elsewhere clearly demonstrate a correlation between elevated blood levels and proximity to the smelting plants.

The data presented do not support the confident assertion that "no harmful effects had been found" in children with elevated blood lead levels. Detection of low level lead toxicity required a sophisticated battery of specialized testing for learning disabilities, behavioural anomalies, disturbances in co-ordination and in motor nerve conductivity velocity.

The author is also requested to provide data on air lead levels in the smelting plants studied.

MARTIN (U.K.)

The observation "no harmful effects have been found" does not necessarily mean that no harmful effects occurred. The surveys were carried out by local authority public health departments and any abnormality, such as a raised blood lead level in a child, had to be referred to the child's family doctor with, if necessary, a suggestion that he might seek the advice of a (named) consultant paediatrician. Thus in the Tower Hamlets survey all such children needing further investigation were referred to a paediatrician at the Great Ormond Street Hospital for Sick Children. In the Tower Hamlets survey a special investigation of intelligence and behavioural defects was undertaken by Landsdown and his colleagues and reported in The Lancet (1974, i, 538)

The measurement of air lead level in the works was beyond the scope of the investigation.

CHARLTON (U.K.)

What examinations were undertaken to investigate the transport of lead in clothing? Have the lockers in which worker's clean clothing is stored been checked? Is there any relationship between the distance workers have to travel home and the lead transported home.

MARTIN (U.K.)

Any follow-up of employees was the responsibility of, and was undertaken by the Department of Employment Factory Inspectorate and by the works doctor. Lead dusts were found in the underclothing, socks, and on the boots of workers and also in their cars. Changing and washing facilities in the works were checked.

It was noted that some of the workers, whose families were affected, lived some miles from the works.

DIEHL (Federal Republic of Germany)

Dr. Martin's observation that rather high lead exposure in the vicinity of lead works is not associated with any symptoms of lead intoxication of the population is in full agreement with other results presented at this conference. De Graeve has reported on a population group in Belgium which is exposed to elevated lead levels in drinking water and McNeil has studied the population of Smeltertown, Texas, which has been exposed to high environmental lead levels for 3 generations. In no case were indications of lead poisining observed.

I am interested in this problem from the viewpoint of food legislation. In some countries (the Federal Republic of Germany for instance) very strict legislation is now in preparation which sets very low limits for lead, mercury, cadmium and arsenic in foods - with the argument that this is necessary to protect the health of the consumer. After 4 days of this conference, I do not have the impression that such measures are justified in view of recent research results. I have already mentioned the case of lead. Turning to mercury I would like to recall Bernstein's paper at this conference indicating that a Canadian population group consuming very much fish with mercury contents of up to 4.4 ppm is not showing any symptoms of mercury intoxication. I was glad to hear from den Tonkelaar that no necessity is seen in the Netherlands to limit fish consumption although much fish is consumed there, and although some of the fish caught in Dutch waters contains over 0,5 ppm mercury - the limit set in the USA. As to cadmium, Lorke has found no toxicity of a dirt containing 30 ppm Cd in subchronic studies with rats and dogs. All this information indicates to me that no hasty legislation is necessary to set limits for these elements in the general food supply. I would like to hear Dr. Martin's comment on the attitude taken by the UK authorities in this respect.

MARTIN (U.K.)

Detailed surveys involving many thousands of analyses have been made of the lead, cadmium and mercury content of foods in the UK and the results published by the Ministy of Agriculture Fisheries and Food. These yielded information on the respective content of individual items of the diet and on the total diet of the average man. Britain is not a big fish-eating country and in the case of mercury, investigations of heavy fish-eaters did not show levels of mercury in the body which could be judged potentially dangerous.

Food contamination in the UK is controlled by, for instance, the Lead in Food Regulations or, in other cases, by measures which prevent the sale of shellfish from polluted areas. While the contamination of food in manufacture of as a result of spraying in agriculture can be controlled, it is not practicable to limit the natural content of foods. Thus a retailer could not be expected to know the mercury content of the individual fish or the cadmium content of the kidneys or liver he sells for human consumption.

VARIAZIONI DELL'ATTIVITA' ALA-DEIDRATASICA ERITROCITARIA IN RAPPORTO ALL'ETA' ED AL SESSO IN SOGGETTI NON PROFESSIONALMENTE ESPOSTI A PIOMBO

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RIASSUNTO

Lo studio dell'attività ALA-deidratasica (ALAD) eritrocitaria di soggetti sani, non professionalmente esposti a contatto con piombo ed abitanti in una grande città industrializzata, ha dimostrato che questa attività enzimatica diminuisce progressivamente con l'aumentare dell'età; questo fenomeno é interpretato come espressione di un progressivo incremento dell'accumulo di piombio nell'organismo, con il passare degli anni.

Nei soggetti di sesso femminile il decremento dell'attività ALAD eritrocitaria é meno evidente e più tardivo che nei soggetti di sesso maschile.

ABSTRACT

A study of ALA-dehydratase (ALAD) activity in the red blood cells of healthy subjects who are not exposed to lead in the course of their work and who live in an industrial city shows that this enzyme activity diminishes gradually with age. This phenomenon is taken to reflect the gradual increase in the amount of lead accumulated in the organism over the years.

In female subjects, the decrease in ALAD activity in the red blood cells is less evident and slower than in male subjects.

1. Introduzione

E' stato ormai estesamente dimostrato che l'attività ALA-deidratasica (ALAD) eritrocitaria é elettivamente inibita dal piombo; questa inibizione é particolarmente cospicua nella intossicazione conclamata da piombo, ma é già dimostrabile anche in occasione di modesti incrementi dello assorbimento di questo metallo: una parziale inibizione di questa attività enzimatica, per esempio, é stata documentata in soggetti che vivono in vicinanza di fonderie di piombo (Secchi e coll. [1], [2], Nordman e coll. [3]) e persino nella popolazione generale di città fortemente industrializzate (Secchi e coll. [1], [2] , Henberg e Nikkanen [4], Hernberg e coll. [5]). L'inibizione dell'ALAD eritrocitaria é perciò considerata un sensibile parametro, atto a documentare gli effetti sull'organismo umano dell'inquinamento ambientale da piombo. In questa ricerca abbiamo studiato il comportamento dell'attività ALAD eritrocitaria in soggetti abitanti in una grande città industrializzata, non esposti professionalmente a piombo, suddivisi secondo il sesso e di età progressivamente crescente, al fine di indagare se le variazioni di questa attività enzimatica potessero documentare un eventuale progressivo accumulo di piombo nell'organismo, che gradualmente aumenta con il progredire dell'età.

2. Materiali e metodi

La ricerca é stata condotta su 168 soggetti viventi in Milano: di essi 89 erano di sesso maschile e 79 di sesso femminile. La casistica é stata suddivisa per sesso e per età e sono stati costituiti i seguenti gruppi: sangue del funicolo ombelicale (11 maschi - 10 femmine); soggetti di età compresa:fra 3 e 6 anni (9 maschi - 9 femmine); fra 8 e 11 anni (15 maschi - 15 femmine); fra 12 e 15 anni (6 maschi - 7 femmine); fra 20 e 40 anni (30 maschi - 20 femmine); fra 60 e 90 anni (18 maschi - 18 femmine). Tutti i soggetti esaminati erano apparentemente sani, non risultavano esposti a contatto con piombo per motivi professionali né al momento dell'esame né in precedenza, non erano forti consumatori di alcoolici. Il campione di sangue per la determinazione dell'attività ALAD eritrocitaria (0,8 ml) é stato ottenuto in occasione di prelievi di sangue per scopi medici (screening di massa, ecc..). L'attività ALAD eritrocitaria é stata determinata secondo il metodo Europeo Standardizzato dalla C.C.E.(1973).

3. Risultati

I risultati sono esposti nella tabella I. L'esame dei risultati dimostra che nei primi 3 gruppi presi in considerazione (sangue del funicolo ombelicale, soggetti di età compresa fra 3 e 6 anni e fra 8 e 11 anni) l'attività ALAD eritrocitaria si mantiene del tutto costante e che non esistono differenze fra i due sessi. Nei soggetti di sesso maschile si osserva successivamente un progressivo decremento dell'attività ALAD eritrocitaria con il progredire dell'età: esso é modesto nel gruppo di soggetti di età compresa fra 12 e 15 anni, diviene più manifesto nei soggetti di età compresa fra 20 e 40 anni, mentre nei soggetti di età compresa fra 60 e 90 anni l'attività enzimatica é ridotta del 50% ed oltre rispetto ai valori osservati nell'età infantile. Nei soggetti di sesso femminile, invece, i valori dell'attività enzimatica si mantengono praticamente a livelli costanti anche nei gruppi di età compresa fra 12 e 15 anni e fra 20 e 40 anni ed un decremento di essi si osserva solo nei soggetti di età compresa fra i 60 e 90 anni. Anche in questo gruppo il decremento della attività enzimatica é certamente inferiore a quello osservato nel gruppo corrispondente di soggetti di sesso maschile.

4. Discussione

Un'analisi statistica dei risultati ottenuti dimostra che l'attività ALAD eritrocitaria subisce con il progredire dell'età una progressiva riduzione. Questo fenomeno é chiaramente evidente nella casistica di sesso maschile e si manifesta già a partire dal 3° quinquennio di vita. Esso sembra indicare l'effetto di un progressivo aumento dell'accumulo di piombo nell'organismo umano, con il passare degli anni. Le ricerche di Beasley e coll. [6] hanno, d'altronde, dimostrato che in soggetti sani anche i livelli di piombemia si innalzano gradualmente con l'aumentare dell'età (é tuttavia da rilevare che altre indagini, condotte in epoca precedente, non avevano evidenziato questo fenomeno - Hofreuter e coll. [7], Hammond [8]). La progressiva riduzione dell'attività ALAD eritro

Tabella I - VARIAZIONI DELL'ATTIVITA' ALA-DEIDRATASICA ERITROCITARIA IN RAPPORTO ALL'ETA' ED AL SESSO IN SOGGETTI NON PROFESSIONALMENTE ESPOSTI A PIOMBO.

	SANGUE DEL FUNICOLO OMBELICALE	3 - 6 anni	8 - 11 anni	12 - 15 anni	20 - 40 anni	60 - 90 anni
N° dei soggetti	11	9	15	6	30	18
SESSO MASCHILE						
ALAD U.I./L G.R.	42,25	45,40	45,32	38,45	32,18	20,46
N° dei soggetti	10	9	15	7	20	18
SESSO FEMMINILE						
ALAD U.I./L G.R.	41,09	44,46	44,66	46,53	42,10	34,84

ANALISI DELLA VARIANZA PER UN MODELLO MISTO, ESSENDO RANDOMIZZATA LA VARIABILE ETA' E FISSA LA VARIABILE SESSO.

SORGENTE DELLA VARIAZIONE	SS	df	MS	F	P
SESSO	1621,39	1	1621,39	31,23	< 0,001
ETA'	6831,67	5	1366,33	4,15	< 0,10
INTERAZIONE SESSO-ETA'	1645,76	5	329,15	6,39	< 0,01
ERRORE	8099,18	156	51,92		

citaria con l'aumentare dell'età non é altrettanto evidente nella casistica di sesso femminile da noi esaminata: in questa casistica il fenomeno é ben evidente solo a partire dalla 6° decade di vita. Ne deriva che nei soggetti di sesso femminile, nell'età adulta, l'attività ALAD eritrocitaria risulta più elevata che nei soggetti di sesso maschile, come d'altronde già constatato da Haeger Aronsen e coll.[9], da noi stessi [2] e da Valloton e coll [10]. La casistica da noi studiata é costituita da soggetti abitanti nella stessa città e pertanto si può ritenere che tutti i soggetti esaminati fossero esposti allo stesso assorbimento di piombo per via inalatoria. Le differenze da noi riscontrate fra i due sessi potrebbero pertanto essere attribuite, in via di ipotesi, ad un diverso assorbimento di piombo per via digestiva, per differenti abitudini alimentari fra i due sessi. Questa ipotesi non ci sembra sufficiente ad interpretare i risultati ottenuti, in quanto le prime differenze fra i due sessi si osservano già nel 3° quinquennio di vita, quando le abitudini alimentari non sono in pratica differenti e, soprattutto, non esiste ancora quel maggior consumo di vino e di alcoolici nel sesso maschile che, secondo recenti dati (Moore e coll.[11], Krasner e coll.[12], Secchi e Alessio [13]), potrebbe giustificare una più spiccata inibizione della attività ALAD eritrocitaria. Altri fattori debbono pertanto essere in gioco: essi potrebbero collegarsi alle emorragie periodiche del sesso femminile, tali da determinare nella donna l'esistenza di una popolazione eritrocitaria costituita da cellule più giovani. E' d'altra parte noto come l'attività ALAD eritrocitaria sia particolarmente elevata nelle cellule rosse più giovani (Battistini e coll.[14]). Questa seconda ipotesi può essere convalidata dal fatto che le prime differenze fra i due sessi, per quanto riguarda i livelli di attività ALAD eritrocitaria, si osservano a partire dal 3° quinquennio di vita e che invece dalla 6° decade di vita in poi, l'attività ALAD eritrocitaria subisce un decremento anche nei soggetti di sesso femminile.

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DISCUSSIONE

HERNBERG (Finlandia)

Dispone Lei di misurazioni di piombemia per il Suo gruppo? Il ricorso a questi dati Le avrebbe forse risparmiato molte congetture circa l'accumulazione di piombo con l'avanzare dell'età. Come Lei certamente saprà, il collega S. Tola ha dimostrato che il rapporto piombemia/ALA-D è in pratica quasi sempre il medesimo (Work-Envir. Health 10, suppl.1, 1973).

ALESSIO (Italia)

I livelli di piombo nel sangue sono stati determinati nei gruppi di età compresa fra 20 e 40 anni; i livelli di piombemia sono risultati significativamente più elevati nei soggetti di sesso maschile.

Per quanto riguarda gli altri gruppi di età purtroppo non dispongo ancora dei dati riguardanti i livelli di piombo nel sangue.

CARNOW (U.S.A.)

Lei fa notare una differenza di sesso all'età di 12 anni. Ha Lei preso in considerazione il fumo quale possibile spiegazione di questo fenomeno?

E' stato dimostrato che i fumatori di sigarette hanno in genere un tenore di piombo più elevato nel sangue per effetto dell'assorbimento dal fumo.

ALESSIO (Italia)

In questa ricerca non è stata valutata l'influenza del fumo di tabacco sull'attività ALAD eritrocitaria.

HINE (U.S.A.)

Il nostro gruppo ha studiato il tenore in piombo nel sangue, nei tessuti molli e nelle ossa di persone da 1 a 90 anni. I livelli più elevati di piombo nei tessuti sono stati riscontrati nei soggetti compresi tra i 30 e i 70 anni, mentre in seguito si é notata una diminuzione.

L'inibizione più elevata dell'ALAD nelle vostre serie tra i 60-90 anni avrebbe luogo a più basse concentrazioni di piombo. Si renderebbe pertanto necessario un altro fattore al fine di spiegare la diminuzione dei valori dell'ALAD da voi osservati.

ALESSIO (Italia)

Poichè non dispongo ancora dei dati riguardanti i livelli di piombo nel gruppo da Lei indicato non posso attualmente escludere o confermare le Sua interessante ipotesi.

SIGNIFICATION DES INDICATEURS BIO-ANALYTIQUES DE L'EXPOSITION AU PLOMB AU SEIN D'UNE POPULATION NON PROFESSIONNELLEMENT EXPOSEE

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**avec l'aide technique de Mmes M. Milissen-Vande Riviere et
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RESUME

Au cours d'une enquête épidémiologique effectuée au sein d'une population non sélectionnée et non professionnellement exposée, les auteurs ont étudié la signification de quelques indicateurs bio-analytiques de l'exposition au plomb: plombémie, ALAD et ALAU. Après avoir décrit brièvement les techniques utilisées et discuté les résultats obtenus, ils arrivent aux conclusions suivantes:

- la mesure de l'ALAD, paraît un indicateur sensible de l'exposition non professionnelle au plomb tandis que la mesure de l'ALAU semble devoir être écartée;

- la mesure de la plombémie reste délicate et insuffisamment reproductible pour être utilisée dès à présent mais il existe indiscutablement une corrélation valable entre la plombémie et l'activité ALAD.

ABSTRACT

During an epidemiological survey on sections of the population which had not been specially selected and were not occupa-

tionally exposed to lead the authors investigated the value of some bio-analytical indicators of exposure to lead: lead in the blood, ALAD and ALAU. After giving a brief description of the techniques employed and discussing the results obtained, they come to the following conclusions:

- ALAD measurement appears to be a sensitive indicator of non-occupational exposure to lead, but ALAU measurement must, it would seem, be eliminated;

- the measurement of lead in the blood remains difficult and is not reproducible enough to be used at present, though there is unquestionably a valid correlation between blood lead level and ALAD activity.

Introduction

Bien que le saturnisme soit connu depuis la plus haute antiquité, l'intérêt des toxicologues pour le plomb s'est réveillé recemment en raison des multiples pollutions dues aux conditions techniques de la vie moderne.

Les principaux indicateurs bio-analytiques actuellement proposés pour l'exposition non professionnelle au plomb sont la mesure de la plombémie, celle de l'activité de la déhydratase de l'acide deltaaminolévulinique dans le sang (ALAD) et celle de l'excrétion de l'acide deltaaminolévulinique dans les urines (ALAU). Les problèmes techniques posés par le dosage de la plombémie ne sont pas encore entièrement résolus (Berlin et coll.) (1) particulièrement en ce qui concerne les faibles teneurs que l'on peut rencontrer dans les expositions à la pollution saturnine de l'environnement.

Au cours de la présente enquête épidémiologique, les auteurs se sont attachés à dégager la signification de la mesure de l'ALAD, de l'ALAU et celle de la plombémie dans l'estimation des risques sanitaires que comporte la pollution de l'environnement par le plomb.

Population examinée

La population choisie n'est pas professionnellement exposée au plomb et peut être considérée comme étant en bonne santé. Au cours de notre enquête qui s'est étalée de fin 1972 à début 1974, cette population a été examinée deux fois.

1. Lors de la première série d'examens, nous avons effectué le dosage de l'ALAD chez 143 personnes et le dosage de l'ALAU chez 140 d'entr'elles.

   Nous avons classé cette population en 106 hommes, 37 femmes, 66 fumeurs, 69 non fumeurs ainsi que par âge.
2. La deuxième série d'examens a porté pour la quasi totalité sur les mêmes personnes réexaminées à environ un an d'intervalle. Cette seconde série a comporté le dosage de l'ALAD et de la plombémie et nous n'avons retenu que les 124 personnes pour lesquelles nous avons pu obtenir ces deux mesures sur le même échantillon.

Ces 124 personnes se répartissent en 86 hommes, 38 femmes, 60 non fumeurs et 63 fumeurs.

Matériel et Méthodes

1. Dosage de l'ALAU

Pour ce qui est de l'excrétion de l'ALAU, les auteurs ont utilisé la méthode classique de DAVIS.

2. Dosage de l'ALAD

Lors de la première série d'examens, nous avons utilisé une technique dérivée de celle de BONSIGNORE (2).
Lors de la deuxième série, nous avons suivi strictement la méthode européenne standardisée décrite en détail par ailleurs (3). Les principales différences avec notre technique initiale sont les suivantes :

- remplacement du TRITON X 100 comme agent hémolysant par eau distillée;
- incubation à pH 6,4 au lieu de pH 7;

Afin d'éviter toute confusion dans l'expression des résultats, nous donnons, ci-après, la formule du calcul des unités que nous avons utilisées :

$$\text{Unités=micromôles ALA/min/L RBC} = \frac{E \times 2 \times 35 \times K}{\text{Hct en \%} \times 60 \times 0.062 \times 0.01}$$

E : extinction mesurée
Hct : hématocrite
K : facteur d'étalonnage du spectrophotomètre = 1.099

3. Mesure de la plombémie

Pour ce qui est de la mesure de la plombémie, nous avons utilisé une technique d'absorption atomique sans flamme. Il serait également hors de propos d'entrer ici dans les détails de cette technique dont nous ne citerons que les principales étapes.
Appareil utilisé : AA Perkin Elmer équipé du four graphite (avec tube spécial pour solution organique) et du "Deuterium-Corrector" avec "Gazstop".
L'échantillon (sang total hépariné) est dilué 6 fois dans une solution à 0,5% de TRITON X 100. Après hémolyse, on

injecte 10 microlitres de cette dilution dans le four graphite. On réalise l'étalonnage interne par addition de quantités connues d'une solution standard de plomb.

Résultats et Discussion

Afin de faciliter la compréhension et l'interprétation de nos résultats, nous les avons présentés sous forme de tableaux et graphiques.

1. Dosage de l'ALAU

Le tableau 1 résume les résultats de nos dosages d'ALAU. On peut en tirer les quelques considérations suivantes :

- toutes les valeurs de l'ALAU que nous avons déterminées sont basses : elles se situent toutes en-dessous de 5 mg par gr de créatinine.
- l'analyse statistique n'a montré aucune corrélation significative entre les mesures d'ALAD et d'ALAU dans les divers groupes de population considérés.

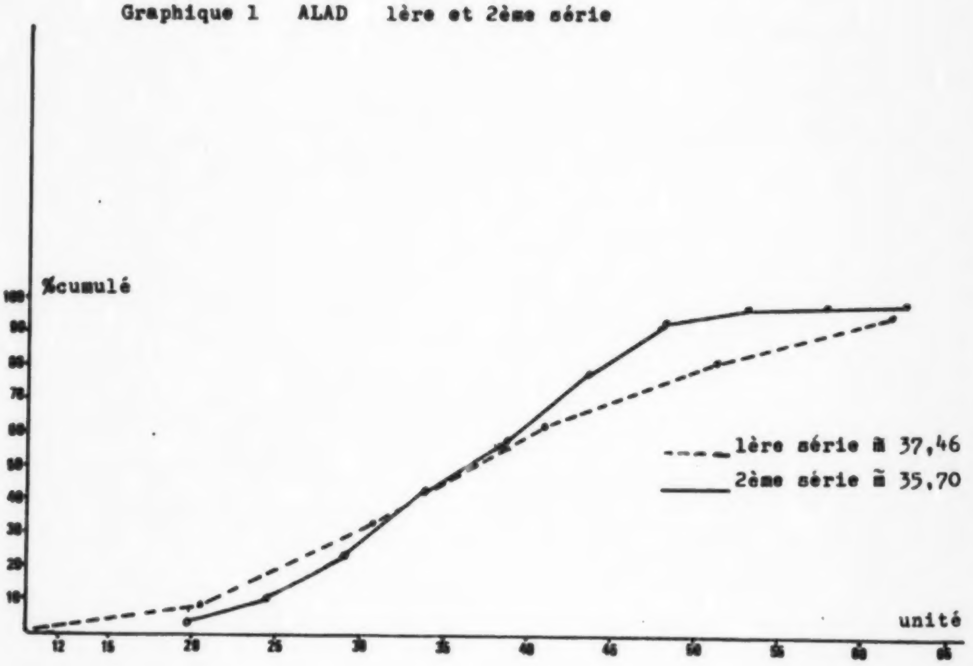
En conclusion, il nous a paru que le dosage de l'ALAU ne semblait pas représenter un indicateur bio-analytique sensible de l'exposition non professionnelle au plomb et c'est la raison pour laquelle nous avons abandonné cette mesure.

TABLEAU 1.

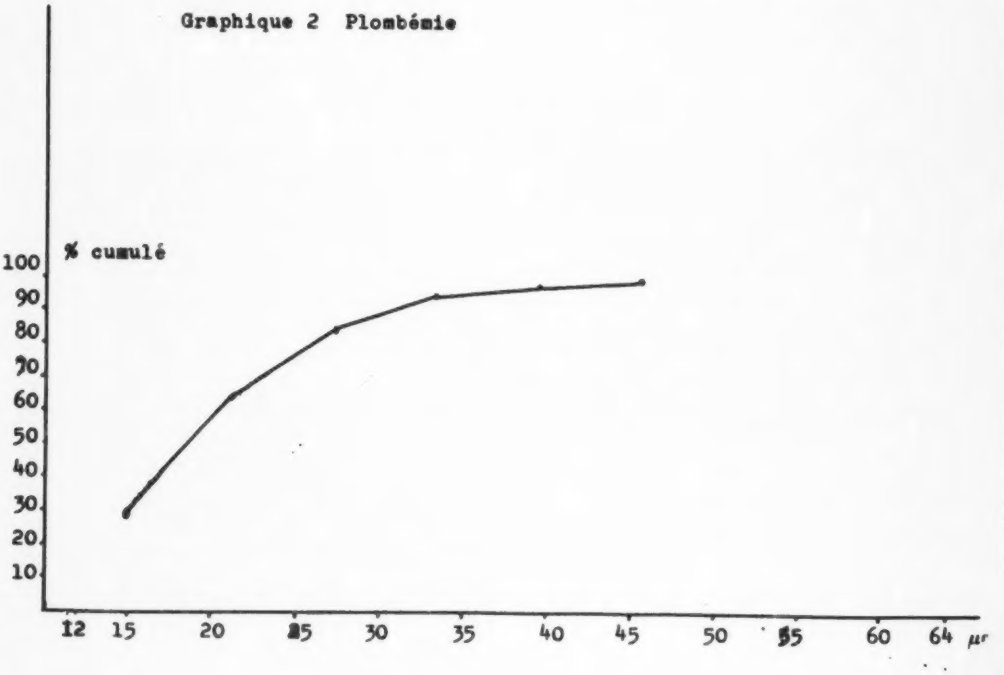
Répartition des mesures de l'excrétion de l'acide deltaaminolévulinique dans les urines. (exprimées en mg d'acide deltaaminolévulinique par gramme de créatinine).

Milligrammes d'A.L.A. par gramme de créatinine	Pourcentage	Pourcentage cumulé
<1	26 %	26 %
1 à 2	50 %	76 %
2 à 3	20,3 %	96,3 %
3 à 4	2,5 %	98,8 %
4 à 5	1,2 %	100 %

Graphique 1 ALAD 1ère et 2ème série



Graphique 2 Plombémie



2. Dosage de l'ALAD - Comparaison entre les résultats obtenus lors des deux séries d'examens.

Le graphique 1 illustre cette comparaison. Rappelons qu'il s'agit des mêmes personnes examinées à environ 1 an d'intervalle. Bien que la technique utilisée n'ait pas été strictement la même, on peut remarquer que les résultats sont cependant assez proches : les moyennes ont été de 37,46 et de 35,7 unités, respectivement pour les première et deuxième séries d'examens.

Cependant, il faut souligner que l'adoption de la méthode européenne standardisée a permis d'obtenir une moindre dispersion des résultats : 90% des résultats obtenus sont compris entre 19,5 et 48 unités alors que dans la première série, ils s'étalaient de 10 à 57 unités.

Nous avons également comparé les valeurs individuelles obtenues à 1 an d'intervalle : le coefficient de corrélation est de + 0,56. (valeur limite + 0.28 pour p=0.01).

On pourrait donc penser, bien qu'il soit nécessaire de le confirmer par des expériences ultérieures, que la valeur ALAD est assez stable chez un même individu.

Ajoutons que lors d'un essai réalisé chez 6 volontaires de notre laboratoire, le coefficient de variation de la mesure ALAD, déterminée une fois par semaine pendant 4 semaines consécutives, a été de 6,29%.

3. Mesures de la plombémie

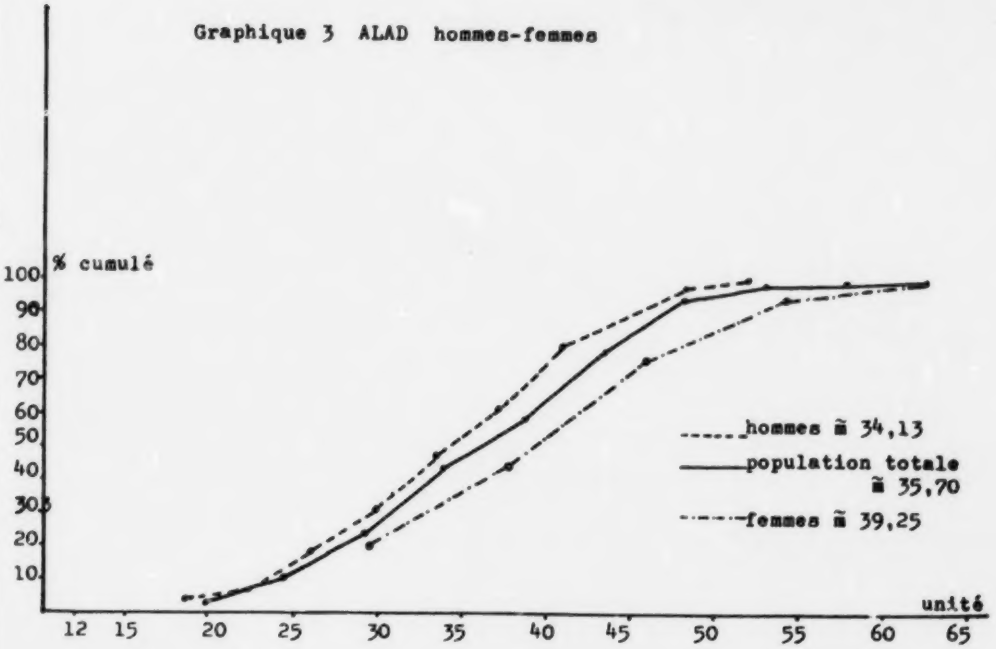
Le graphique 2 résume les résultats de nos 124 déterminations.

Remarquons que :

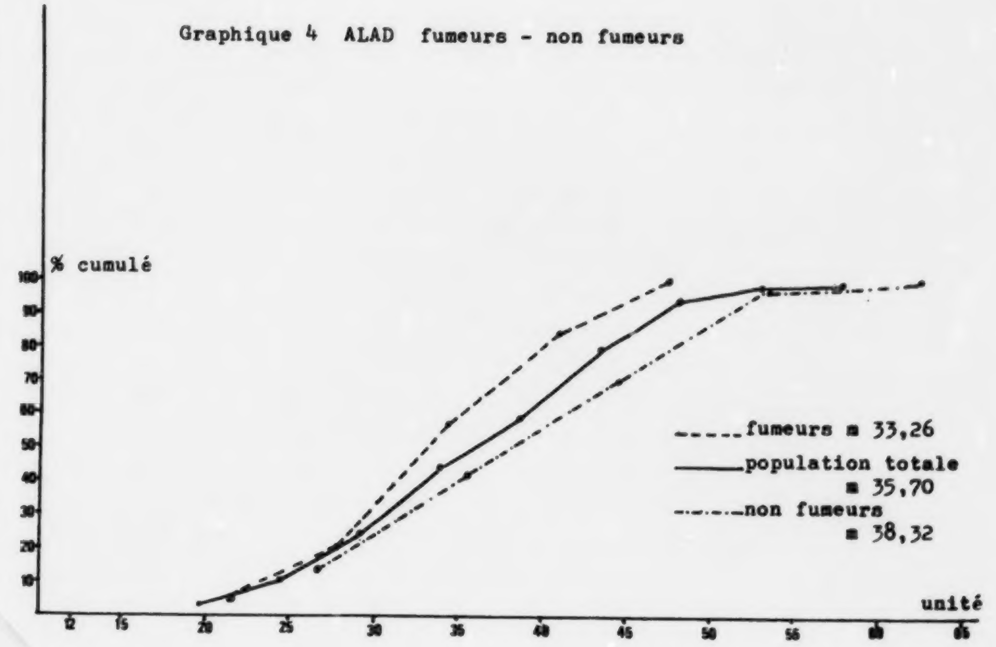
- 60% des plombémies se situent en-dessous de 20µg %
- 90% en-dessous de 31µg %
- 98% en-dessous de 40µg %.

Ces valeurs se rapprochent assez fort des valeurs guides proposées par ZIELHUIS (4). On pourrait en conclure que la population que nous avons examinée ne semble pas être exposée à un risque appréciable au point de vue exposition au plomb.

4. Comparaison des mesures ALAD entre hommes et femmes
   Le graphique 3 illustre bien la différence entre ces 2 groupes : les femmes ont une activité ALAD nettement plus élevée que les hommes. Nous avons utilisé le test t pour évaluer le degré de signification de la différence entre les 2 moyennes. Le calcul de t donne : 3.061 : la valeur limite de t au niveau 0.01 étant 2,62, nous pouvons en déduire que la différence est significative.
5. Comparaison des mesures ALAD entre fumeurs et non fumeurs
   Le graphique 4 montre la différence entre ces 2 groupes : les fumeurs ont manifestement une activité ALAD plus basse. Le test t dont le calcul donne la valeur 3.275 indique également une différence significative entre les 2 moyennes.
6. Activité de l'ALAD en rapport avec l'âge
   L'analyse statistique a montré qu'il existait une diminution progressive de l'ALAD avec l'âge. Cette diminution s'est manifestée dans les deux séries d'examens mais elle n'est significative qu'au niveau p=0.05 : les coefficients de corrélations calculés ont été respectivement pour les première et deuxième séries de -0,19 et -0,186 (valeur limite pour p 0.05=-0,17).
7. Corrélation entre l'activité ALAD et la plombémie
   Le tableau 2 nous montre ces corrélations entre les divers groupes. L'examen de ce tableau nous permet les considérations suivantes :
   a. Pour les mesures d'ALAD, nous retrouvons les différences signalées entre les hommes et les femmes d'une part, les fumeurs et les non fumeurs d'autre part.
      Corollairement, les plombémies sont plus élevées chez les hommes que chez les femmes et chez les fumeurs que chez les non fumeurs. Cependant, l'analyse statistique montre que ces différences sont moins significatives que pour les mesures de l'ALAD. Ceci est dû à une dispersion relativement plus grande pour les mesures de plombémie.



Graphique 3 ALAD hommes-femmes



Graphique 4 ALAD fumeurs - non fumeurs

TABLEAU 2.

Corrélation entre les moyennes ALAD et Plombémie dans les divers groupes de population.

	Nombre de sujets	ALAD Unités		Plombémie µg %		Coefficient de corrélation r	Valeur limite de r pour p=0.01
Population totale	124	35,70		20,69		-0.39	-0.23
Hommes	86	34,13			21,74	-0.40	-0.28
Femmes	38		39,25	18,31		-0.26	-0.41
Fumeurs	63	33,26			22,17	-0.51	-0.32
Non fumeurs	60		38,32	19,23		-0.21	-0.33

b. Pour les mesures d'ALAD et de plombémie, on observe une corrélation négative. Cette corrélation est significative au niveau p=0.01 pour la totalité de la population examinée, pour le groupe des hommes et celui des fumeurs. Elle est moins significative pour les autres groupes.

L'examen du tableau 2 montre clairement que nous n'avons une corrélation significative entre ALAD et plombémie que lorsque la moyenne ALAD du groupe considéré est inférieure à la moyenne générale et lorsque la moyenne de la plombémie est par contre supérieure à la moyenne générale. Les difficultés techniques de la mesure de la plombémie, surtout pour les valeurs basses, pourraient expliquer ce fait.

La mesure de l'ALAD nous paraît donc être un indicateur bio-analytique valable et sensible de l'exposition saturnine d'une population non professionnellement exposée au plomb, surtout que l'on dispose actuellement d'une méthode bien standardisée permettant de comparer les résultats obtenus dans divers pays.

Conclusions générales

Bien que cette enquête ait été très limitée et qu'elle doive être étendue à d'autres groupes de population, notamment à des groupes critiques, il nous semble que l'on puisse en tirer les quelques conclusions provisoires suivantes :

1. Dans l'état actuel de la pollution de l'environnement par le plomb et pour la population que nous avons examinée, il ne paraît pas y avoir de risque sanitaire appréciable si l'on se réfère aux limites de ZIELHUIS.
   Cependant, une surveillance attentive nous semble devoir être instaurée, particulièrement dans des régions à risques accrus.
2. La mesure de l'ALAU ne paraît pas devoir être retenue comme un indicateur bio-analytique valable de l'exposition au plomb en dehors de l'exposition professionnelle.
3. Par contre, la mesure de l'ALAD semble être un indicateur sensible et éventuellement utilisable de cette exposition. Toutefois, il est encore prématuré de dégager, dès à présent, des critères à partir du dosage de l'ALAD.
   Des programmes d'intercomparaison effectués en utilisant une méthode bien standardisée et étendue à divers pays et régions devraient permettre d'aboutir à l'établissement de critères.
4. Le choix d'un indicateur comme l'ALAD se justifie actuellement tant que la technique de la mesure de la plombémie n'aura pas acquis la reproductibilité et la fiabilité indispensables pour lui rendre le rôle de premier témoin de l'imprégnation saturnine.

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DISCUSSION

HERNBERG (Finlande)

Je voudrais faire deux observations au sujet de votre intéressante étude: premièrement, en 1972, mes collègues MM. Tola, Nikkanon et moi-même avons étudié une population d'environ 1.500 personnes. Nous avions trouvé qu'en normalisant vis-à-vis de la plombémie,ni l'âge, ni le tabagisme n'avaient pratiquement d'effet significatif sur ALA-D. De plus nous avons constaté que le rapport PbB ALA-D était le même pour une exposition professionnelle nouvelle, pour un taux constant d'exposition et aussi longtemps après la fin de l'exposition. Ainsi l'ALA-D reflète la teneur en plomb du sang et les différences que vous avez trouvées peuvent très probablement s'expliquer par des PbB différents. Les résultats auxquels je me suis référé ont été publiés il y a un an dans Work-Environment-Health (Tola, S., Wk-Environ. Hlth 10, suppl. 1, 1973). En second lieu je pense que la raison pour laquelle vos valeurs t étaient plus significatives pour ALA-D que pour PbB est que vous ne devriez pas utiliser la statistique t pour la distribution log. normale d'ALA-D. Si, au lieu de cela, vous aviez calculé le logarithme des valeurs ALA-D, je pense que le test t aurait donné le même résultat que pour PbB. J'estime donc que l'explication de la divergence apparente est simplement que vous avez employé une méthode statistique inadequate.

BRUAUX (Belgique)

1. Pour ce qui est de la première observation du Dr. Hernberg, je suis d'accord avec lui sur le fait que les différences que nous avons observées dans les mesures de l'ALA-D suivant le sexe, l'âge, le tabagisme sont associées à des différences dans la plombémie. Le tableau 2 montre d'ailleurs clairement la corrélation négative existant entre les valeurs ALA-D et celles de la plombémie. Ce que nous avons voulu montrer dans ce travail c'est que la mesure de l'ALA-D est plus sensible et plus précise que celle de la plombémie. Ceci explique que les différences statistiques entre les différents groupes soient plus significatives pour les mesures de l ALA-D que pour celles de la plombémie.

2. Quant à la deuxième observation, je puis dire que le test t a été appliqué sur les valeurs logarithmiques de l'ALA-D ce qui rend, je pense, l'application de ce test valable pour évaluer la différence entre les moyennes.

DIAMETRE ERYTHROCITAIRE MOYEN CHEZ LES ADULTES HABITANT UNE VILLE AVEC UNE ATMOSPHERE POLLUEE PAR LE PLOMB INORGANIQUE DE SOURCE INDUSTRIELLE

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RESUME

Nous nous sommes proposés d'examiner le comportement du diamètre érythrocytaire moyen (DEM; µ) chez les adultes d'une ville dont l'atmosphère est polluée - ville P - par du plomb inorganique de provenance industrielle. 359 sujets non exposés professionnellement au plomb (groupe P) ont été examinés comparativement à deux groupes témoins: le groupe T, comprenant 115 témoins, et le groupe C - de contrôle - comprenant 100 ouvriers professionnellement exposés au plomb mais sans symptômes cliniques.

Chez le groupe P la valeur moyenne ($\bar{x} \pm s$) du DEM était de 7.76 ± 0.64, alors que chez les sujets du groupe T, la valeur moyenne était 7.06 ± 0.49. C'est chez le groupe C que la moyenne était la plus elevée (8.00 ± 0.24). Les différences ont été significatives ($p < 0,01$).

Les aspects des courbes Price-Jones obtenus pour les trois groupes sont caracterisés par la catégorie des dimensions à pourcentage de fréquence maximale,par la baisse du peak, un glissement vers la droite et la présence d'une traîne à droite, allant de T vers P, plus marquée pour C.

Les faits ci-dessus démontrent que l'influence prolongée du contact avec le plomb environnant détermine une tendance vers la

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macrocytose chez la population adulte exempte de signes cliniques.

On considère que l'étude du DEM pourrait constituer une méthode de screening pour le diagnostic de groupe, en cas d'exposition au plomb chez des groupes de population habitant des zones urbaines avec des degrés différents de pollution par le plomb.

ABSTRACT

We decided to examine the behaviour of the mean erythrocyte diameter (MED; μ) in adults living in a town - town P - whose atmosphere is polluted by inorganic lead of industrial origin. 359 subjects not occupationally exposed to lead (group P) were examined and results compared with two control groups: group T, comprising 115 subjects and group C, comprising 100 workers occupationally exposed to lead, but showing no clinical symptoms.

In group P the mean value ($\bar{x} \pm s$) of the MED was 7.76 ± 0.64, whereas in group T subjects the mean value was 7.06 ± 0.49. The mean was highest (8.00 ± 0.24) in group C. The differences were significant ($p < 0.01$).

The Price-Jones curves obtained for the three gorups are characterized by size category at the maximum percentage frequency, by a drop in the peak level, a displacement towards the right and a gradual lengthening of the curve to the right, from T towards P, more marked in the case of C.

The above data show that prolonged contact with lead in the environment tends to cause macrocytosis in an adult population showing no clinical symptoms.

It is thought that the study of the MED might be used as a screening method for group diagnosis in cases of exposure to lead of population groups living in urban areas with various degrees of lead pollution.

1. INTRODUCTION

En partant de la nécessité et de l'actualité de certains test de screening à valeur de diagnostic de groupe dans l'évaluation de l'influence du plomb de l'atmosphère de certaines villes sur la population, nous nous sommes proposés l'étude du comportement du diamètre érythrocytaire moyen (DEM), respectivement la courbe Price-Jones. Nous avons porté notre choix sur ce paramètre de laboratoire clinique pour les raisons suivantes:

a) il peut réfléchir l'affaiblissement d'une fonction physiologique vitale, l'hématopoïèse, qu'une exposition professionnelle au plomb peut, comme on le sait, affecter;

b) on a proposé (1) de considérer que l'altération significative de l'hématopoïèse correspondrait au niveau III de pollution de l'atmosphère des villes, conformément à la classification de l'O.M.S. (2);

c) dans le cas des intoxications au plomb, la plupart des auteurs (3-8 etc.) énoncent l'existence d'une anisocytose à microcytose; d'autres, moins nombreux (9-12) plaident par contre pour l'existence d'un certain degré de macrocytose. Albahari et collab. (13) soutient qu'il n'y a ni mégalocytose ni microcytose, tandis que de Bruin (8) signale l'accroissement du nombre des érythroblastes et quelquefois celui des mégalo - et macroblastes dans la moelle osseuse. Nous n'avons cependant pas rencontré de données concernant la population du milieu urbain faiblement pollué au plomb, et en l'absence des signes cliniques;

d) les résultats de nos travaux antérieurs (14-16; Ghelberg et collab. donnée en cours de publication), où nous avons étudié certains paramètres de laboratoire utiles dans l'appréciation des troubles du processus hématopoïétique chez des groupes d'enfants et d'adultes habitant une ville dans l'atmosphère de laquelle le plomb représente la nocivité potentielle principale, plaident pour l'utilisation de l'indicateur DEM;

e) la détermination DEM présente des avantages aussi bien du point de vue du matériel biologique à examiner, que de celui de l'examen proprement dit et peut être pratiqueé dans les conditions d'un test de screening.

2. MATÉRIEL ET MÉTHODE

En accord avec certaines données de la littérature (17), nous avons jugé utile d'effectuer les analyses sur des groupes plus restreints, mais de manière répétée (1971-1973). Nous avons examiné au total:

- 115 adultes témoins - groupe T - de villes exemptes d'une pollution spécifique au plomb (< 1.o μg Pb/mc air/24 heures);
- 359 adultes non-exposés professionnellement au plomb, mais habitant une ville dont l'atmosphère est polluée par des entreprises de métallurgie non-ferreuse (0.91-22.89 μg Pb/mc air/24 heures, avec une moyenne de 3.98; Ghelberg N.W. Rădulescu N.D., Mihalka St. - données en cours de publication) - groupe P; et
- un groupe de contrôle - C - de la même ville que le groupe P, mais travaillant dans des entreprises de métallurgie non-ferreuse (à la fonte, l'agglomération et le raffinage du plomb) et exempts de symptômes cliniques au moment du prélèvement de sang.

Nous avons déterminé DEM (en μ) de 100 hématies pour chaque sujet, en utilisant de minces frottis de sang périphérique à coloration May - Grünwald - Giemsa; on a aussi tracé les courbes Price - Jones par groupes (T,P,C).

3. RÉSULTATS

Les résultats obtenus sur les groupes de sujets examinés, exprimés sous forme de moyennes arithmétiques et leur paramètres, figurent dans le tableau I.

Il ressort du tableau I que les moyennes DEM vont en croissant, de T (7.06 ± 0.49) à P (7.76 ± 0.64) puis à C où la moyenne est la plus élevée (8.00 ± 0.24). Les différences des moyennes DEM entre les groupes sont significatives (test "t"; $p < 0,05$). Pour une vue plus suggestive sur les valeurs du tableau I, comparativement avec les valeurs moyennes individuelles considérées comme normales (6), nous présentons la figure 1.

Dans la figure 2, nous donnons les courbes de type Price - Jones pour les 3 catégories de sujets: T, P et C.

TABLEAU I

LE DIAMÈTRE MOYEN ÉRYTHROCYTAIRE (DEM) CHEZ LES GROUPES DE SUJÈTS (en μ)

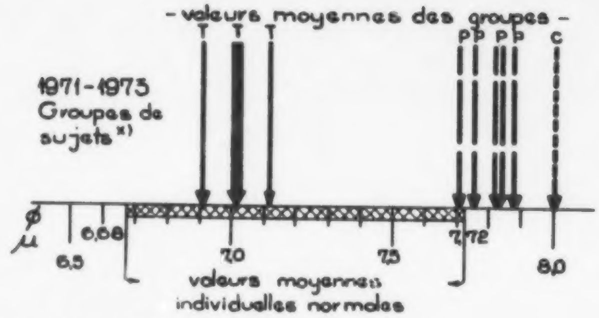
Le groupe x)	n	$\bar{x}$	s	$\bar{x} \pm ts_{\bar{x}}$
T - 1/1971	52	7.08	0.54	7.06 - 7.10
T - 2/1971	10	7.15	0.38	7.13 - 7.17
T - 3/1972	11	6.88	0.49	6.68 - 6.90
T - 4/1972	16	7.02	0.49	7.00 - 7.04
T - 5/1973	26	7.08	0.12	7.07 - 7.08
Total T/1971-1973	115	7.06	0.49	6.98 - 7.14
P - 1/1971	100	7.69	0.71	7.67 - 7.71
P - 2/1972	53	7.75	0.57	7.73 - 7.77
P - 3/1972	37	7.88	0.65	7.86 - 7.90
P - 4/1972	53	7.83	0.57	7.67 - 7.99
Total P/1972	143	7.81	0.59	7.73 - 7.89
P - 5/1973	116	7.74	0.63	7.72 - 7.76
Total P/1971-1973	359	7.76	0.64	7.70 - 7.82
C / 1973	100	8.00	0.24	7.98 - 8.02

Observations x)

T = sujets sans exposition professionnelle au Pb et qui habitent des villes dont la teneur en plomb de l'atmosphère est de < 1.0 μg/24 heures - groupe témoin;

P = sujets sans exposition professionnelle au Pb et qui habitent dans une ville à atmosphère polluée; 0.29 - 12.22 μg Pb/mc air/24 heures.

C = sujets de la ville P et qui travaillent en outre en milieu pollué au Pb; groupe de contrôle.


- valeurs moyennes des groupes -
T
T
T
P P
P P
C
1971-1973
Groupes de sujets x)
Ø
μ
6,5
6,68
7,0
7,5
7,72
8,0
valeurs moyennes individuelles normales

Obs: x) Pour T, P, C voir tableau I.

Figure 1 : Diamètre érithrocytaire moyen en fonction de l'intensité de l'exposition au plomb.

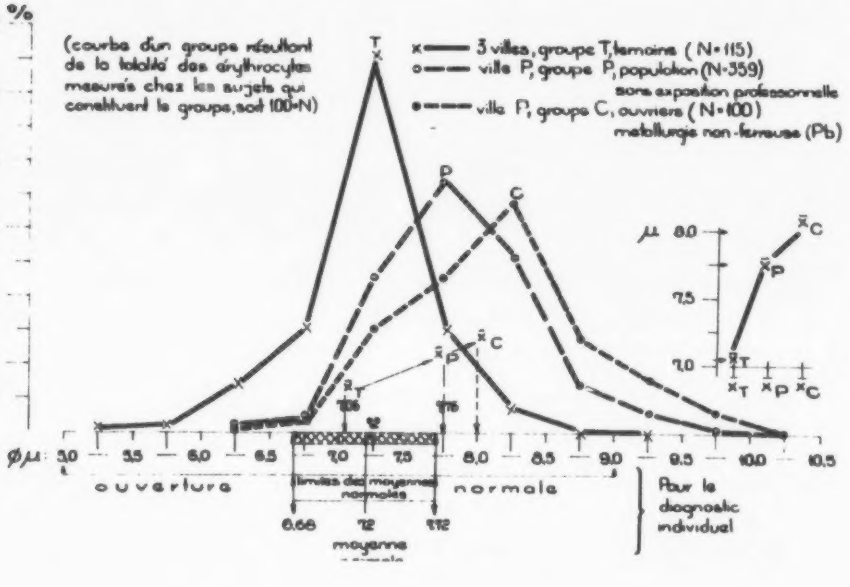


Figure 2 : Courbes Price - Jones des groupes des différentes catégories de sujets.

On peut voir dans la figure 2 le comportement de la courbe T, qui correspond presque parfaitement aux données de la littérature, mais qui diffère catégoriquement des deux autres groupes - P et C - toutes deux déplacées vers la droite et avec une traîne à droite. Cette méthode, celle de la courbe Price - Jones, permet donc elle aussi de voir l'installation d'un certain degré d'anisocytose avec l'augmentation de la fréquence des macrocytes, qui est d'autant plus accentuée que l'exposition au plomb a été plus intense.

Pour souligner certaines relations entre la grandeur DEM et les conditions de l'exposition au plomb, nous présentons quelques détails concernant le groupe C (Ghelberg N.W. et collab. - données en cours de publication). Ce groupe, qui a compris 100 sujets à moyenne de vie de 39.4 ± 7.6 ans, avec une ancienneté moyenne d'exposition au plomb de 14.5 ± 6.8 ans et une moyenne DEM de 8.00 ± 0,24 u, nous l'avons divisé en sous-groupes: qu'ils étaient ou non des fumeurs; en fonction de l'ancienneté du travail en milieu à plomb; et en fonction du lieu de travail. Nous avons calculé les valeurs moyennes du DEM d'après les subdivisions mentionnées. On a constaté des différences significatives ($p < 0,05$) de DEM entre les fumeurs (8.04 ± 0.25) et les non-fumeurs (7.94 ± 0.20) et entre les ouvriers de la fonderie (8.07 ± 0,25), par rapport à ceux de l'agglomérage (7.91 ± 0.20).

On a calculé les coefficients de corrélation totale entre certains caractères du groupe C. Il en est résulté un rapport direct, linéaire, entre l'âge et l'ancienneté dans le travail ($r = 0.470$; $p < 0.01$; ce qui était prévisible), mais aussi entre l'âge et le DEM ($r = 0.211$; $p < 0.05$) et respectivement entre l'ancienneté en milieu à plomb et le DEM ($r = 0.198$; $p = 0.05$).

4. CONCLUSIONS

L'étude du diamètre érythrocytaire moyen (DEM) sur des groupes de population à degrés différents d'exposition au plomb, en tant que polluant de l'environnement (pollution urbaine et celle du milieu de travail) nous a permis les constatations suivantes:

1. Chez les groupes d'adultes du milieu urbain faiblement pollué au plomb de provenance industrielle, le DEM était plus élevé que chez nous propres témoins, dénotant une anisocytose à macrocytose modérée.

2. Chez les ouvriers professionnellement exposés au plomb, mais sans symptômes cliniques, la fréquence des macrocytes était d'autant plus marquée.

3. La détermination DEM et les courbes de type Price - Jones par groupes de sujets, se sont avérés utiles dans le diagnostic de groupe de l'influence du plomb d'environnement sur les groupes de population.

Nous conclurons donc, que l'indicateur étudié se présente comme utile dans son application en tant que test de screening pour l'appréciation de l'influence du plomb sur l'organisme.

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PREVALENCE OF SUBCLINICAL LEAD EXPOSURE IN 761 ASYMPTOMATIC SCHOOL CHILDREN: DENTINE LEAD LEVELS AS A RETROSPECTIVE MARKER

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ABSTRACT

Dentine lead levels were measured from shed deciduous teeth of 761 Philadelphia schoolchildren with no prior history of lead poisoning and residing in two school districts, one considered high risk for lead exposure, and one considered low risk. Black children in public schools from areas of deteriorated housing had marked elevations of dentine lead (mean of 198 µg per gram, in 174 children), with 20 percent of the children having levels in the range associated with toxicity. White children from newer housing had the lowest levels (mean of 41.7 µg per gram, 304 children), but a group of white children from intact housing living near and attending school adjacent to a major lead processor also had elevations of dentine lead (mean of 136 µg per gram, 71 determinations). Lead exposure as defined by dentine lead levels is more serious and widespread than previously acknowledged, and extends to groups other than those traditionally accepted as at risk.

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Recent attempts to study the effects of low level lead exposure have produced conflicting conclusions. While Burde (1) has reported increased incidence of perceptual-motor dysfunction, and behavior disorder, and David (2) reported a higher incidence of increased lead storage in some hyperactive children, the studies of Kotok (3), Albert, et al, (4), and Lansdowne, et al, (5), purported to find no relationship between lesser lead exposure and psychological function. As discussed in the paper by McNeil and Ptasnik on "Evaluation of Long-term Effects of Elevated Blood Lead Concentrations in Asymptomatic Children" presented at this symposium and during the panel on "The Scientific Data Base Required for Decisions to Protect Human Health" two reports of neuropsychological sequelae in high lead children from Smeltertown, Texas, USA draw opposing conclusions as to effects of exposure. These differences may be due in part to problems in experimental design and insensitive psychological outcome measures.

To have validity, a study of the neuropsychological effects of low level lead exposure should meet the following criteria:

(1) It should employ a reliable and valid index of exposure to lead.

(2) It should utilize neuropsychologic instruments sensitive enough to detect subtle impairment, and broad enough to sample performances in at least these areas: perceptual motor, linguistic, and attentional behaviors.

(3) It should identify and scale other factors known to affect neuropsychiatric development which act to confound the effect of lead. Among these are birth status, nutritional history, early rearing quality, and significant illnesses in the subject's history.

(4) It should obtain its subjects in a sampling procedure free of bias.

(5) It should study a sample large enough to allow stratification of other variables that may confound with the effect of lead.

To employ sensitive neuropsychologic instruments at their current stage of development requires the study of children at least six years of age. If exposure has occurred earlier in the life of the child, blood lead concentrations cannot be considered reliable indices of exposure, since they may by this time have returned toward normal. Lead is deposited in bony tissue. The deciduous tooth offers a painless, spontaneous, universal biopsy of bony tissue in children after their sixth year.

In 1962 Altshuler, et al, (6), demonstrated elevations in whole tooth lead in both fatal and non-fatal cases of lead poisoning. Our initial study of lead content of whole teeth from asymptomatic children from the "lead belt" of urban Philadelphia showed that urban children had nearly five times the concentration (mean=51.1 µg/g, N=69) observed in their suburban counterparts (mean=11.0 µg/g, N=40) (7).

Subsequent studies (8,9) showed that lead is concentrated in the secondary dentine adjacent to the pulp, a tissue continuously laid down during the life of the tooth.

When we compared dentine lead levels from nine children who recovered from lead poisoning to healthy children from suburban Boston and Iceland, we found striking differences (10). The mean concentrations were: for lead poisoned children, 601 ± 225 µg/g; for Boston children, 84 ± 56 µg/g, and for Icelandic children, 35 ± 29.8 µg/g. The lowest level observed in a child with overt lead poisoning was 292 µg/g.

Strehlow's studies in the baboon have confirmed that tooth storage of lead is dose related, permanent, and unaffected by chelation (11).

We will present data today that indicates that the study of lead levels in the circumpulpal dentine of shed deciduous tooth provides a reliable index of past exposure, and offers a tool to study large numbers of "normal" children attending school. In addition, our data demonstrate a higher prevalence of exposure than heretofore reported, and also show that children attending school near a lead processor have significant elevations in tooth lead levels.

METHODS

Two Philadelphia school districts, one considered high risk and the other low risk for lead exposure, were identified and entered into the study in 1971. The districts were chosen on the basis of yield, in previous years, of known cases of plumbism.

Characteristics of the Districts

The western half of District 5 is considered within the acknowledged "lead belt" of the city, from which many of the cases of frank plumbism are reported. The population is predominantly black. Although the population is mobile, it tends to remain within the lead belt. The houses are older than 40 years, and many are in a severe state of deterioration. The eastern half of District 5, which is highly industrialized, ends at the Delaware River, and its population is predominantly white. The row houses in this area are old, but in generally good repair. Diagnosed lead poisoning is rare from this sector.

District 8 is the area into which Philadelphia expanded after World War II. The houses are therefore newer, and in generally good repair. The ethnic constitution is predominantly white. In this district, diagnosed lead poisoning is extremely rare.

From each participating school, every child in first grade was given a letter to his or her parents informing them of the nature of the study. When a tooth was presented, the teacher was asked to examine the child's mouth for a fresh socket.

Biochemical Methods

Biochemical methodology is described more fully elsewhere (12). Whole teeth were embedded in self-curing acrylic. A 600 u slice was cut through the center of the tooth. Under a dissecting microscope, the circumpulpal zone of dentine was inserted into a vice to a depth of 300 µ. The free area of the slice was then chiseled away. The circumpulpal dentine, measuring 600 by 300 µ, was dried at 60°C and weighed. After dissolving in 0.1 ml of 70 percent $HClO_4$, the samples were analyzed by Anodic Stripping Voltammetry (Environmental Sciences Associates).

From six public schools and three parochial schools in District 5, and three public and one parochial schools in District 8, samples of interior dust, playground dirt and gutter dirt were obtained. Two schools in suburban Philadelphia away from major traffic patterns or industry were similarly sampled.

Results

Striking differences in lead dentine levels between students in the two school districts were found (Table 1). The highest levels were found in the public schools of District 5, where the student population is predominantly black, but the white parochial schools in District 5 also had elevated dentine lead levels. Children in one parochial school, St. A.'s had marked elevation of tooth lead levels (mean of 136 µg per gram, 71 children) with some individual values among the highest measured in this study.

Figure I shows the cumulative frequency distribution of dentine lead levels for all public school students, and for parochial students attending St. J.'s in District 8 and St. A.'s in District 5. While only 3% of the public and 6.6% of the parochial students of District 8 had dentine levels greater than 100 µg/g, 66% of the black public school students and 43% of the white students of St. A.'s had levels in excess of 100 µg/g. Nineteen percent of the black students from District 5 had dentine levels in the range associated with frank lead poisoning. Eight percent of St. A.'s students also exceeded 300 µg/g. Table II shows the substantial elevations of lead in dust and dirt in and near the schools of District 5.

Table I

Concentration of Lead in Dentine According to School

School	N	Mean Dentine Lead (ug/g)± SEM
District 8		
T.H.	114	42±2.8
B.	59	40±2.9
R.B.P.	69	37±2.6
W.H.L.	62	48±3.6
St. J. #	76	46±2.8
District 5		
N. #	40	66±5.6
V. #	51	92±7.8
St. A. #	71	136±15.6
P.T.	34	120±21.2
P.L.D.	19	131±24.7
J.R.L.	46	208±29.0
G.C.	33	188±30.2
J.E.	29	169±27.5
J.F.	58	191±19.7

# = Parochial Schools

Discussion

These data portray a prevalence of increased body burden of lead in groups considered at risk greater than heretofore reported, and show that

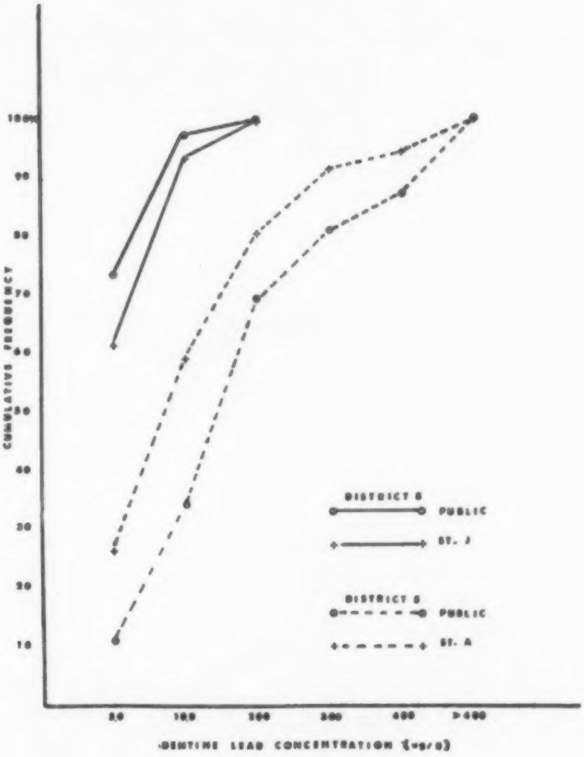

Figure I

Legend: Cumulative frequency distribution of children from District 5 and 8 according to dentine lead level.

Table II

Distribution of Children According to

Dentine Lead Levels by School District and Race

Range of Lead Concentrations (ug/g)

		0-50	51-100	101-200	201-300	301-400	400
	N	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
District 8							
4 Public Schools	304	221(72.7)	74(24.3)	9(2.9)			
White	304						
St.J. parochial	76	47(61.8)	24(31.6)	5(6.6)			
White	76						
Total	380	268(70.5)	98(25.8)	14(3.7)			
District 5							
6 Public Schools	219*						
Black	174	19(10.9)	40(23.0)	62(35.6)	19(10.9)	12(6.9)	22(12.6)
White	7	4(57.1)	2(28.6)	1(14.3)			
Latin	37	9(24.3)	12(32.4)	11(29.7)	4(10.8)	1(2.7)	
Parochial Schools							
N.	40	17(42.5)	17(42.5)	6(15.0)			
white	40						
V.	51	13(25.5)	23(45.1)	13(25.5)	2(3.9)		
white	51						
St.A.	71	19(26.8)	23(32.4)	15(21.1)	8(11.3)	2(2.8)	4(5.6)
white	71						
Total	381*						
Black	174	19(10.9)	40(23.0)	62(35.6)	19(10.9)	12(6.9)	22(12.6)
White	169	53(31.3)	67(39.6)	35(20.7)	10(5.9)	2(1.2)	4(2.4)
Latin	37	9(24.3)	12(32.4)	11(29.7)	4(10.8)	1(2.7)	

Table II

Legend: Public school data was grouped because distribution between schools was similar.

* One child's race was not established.

other groups of children than ordinarily acknowledged have elevated body lead burdens. While 7-12% of high risk groups previously studied are reported to have blood lead levels in the range associated with hazard (>60µg/g) (13), our data show that 66% of black children have elevated dentine lead levels, with 19% in the range found in clinical lead poisoning.

The elevated lead levels in the white parochial school children attending St. A.'s were unexpected, since their homes were in generally good repair. Located one city block away from the playground of St. A.'s is a major manufacturer of paints and lead stearates for plastic fabrications. This finding is consistent with the effects of residence near stationary sources of lead reported by numerous observers (5,14,15), and supports the statement of the National Academy of Science's Task Force on Lead: "The swallowing of lead contaminated dusts may well account in large part for the higher mean blood lead content in urban children, and the rather large fraction whose blood lead content falls in the range of 40-60 µg/100 ml" (16). Our data show that children in intimate proximity to lead processors may experience severe enough exposure to raise their body levels into the range associated with toxicity.

In addition to industrial sources of lead, the entire area of District 5 is subject to extremely heavy automobile traffic. The dust lead levels in the western part of the District, taken at some distance from the lead factories, but yielding concentrations of lead in the same range, suggest that automobile emissions were important sources of dust and dirt lead.

This unexpected finding of elevated dentine lead levels in children in housing of good repair, but who live and attend school close to a major lead processor, as well as the anticipated finding of elevations in children who live in deteriorated housing, suggests that both lead in

Table III

Lead in Individual Environmental Samples

	Lead Concentration (ug/g)		
School	**Interior Dust**	**Playground Dirt**	**Gutter Dirt**
District 8			
T.H.	835	403	2626
R.B.P.	293	444	270
W.H.L.	939	424	1603
St. J.	388	421	1528
District 5			
V.	2838	8683	3031
	929	--	--
St. A.	3074	--	8201
	4947	--	6340
P.T.	1889	--	2392
	3782	--	--
P.L.D.	15680	761	--
	2066	493	--
J.R.L.	5327	2578	2729
	3388	533	--
G.C.	3411	3252	280
	1206	983	--
J.E.	3666	17256	4332
	4416	--	--
J.F.	3206	1207	1515
	1854	--	537
Suburban			
H.	1517	88	1359
	946	67	834
L.	579	38	550
	277	118	714

paint and airborne lead are sources, and that children living in deteriorated housing are in fact being exposed to both sources.

This method of dentine lead analysis now provides a means to conduct large scale retrospective cohort studies in older children considered asymptomatic for lead. If sensitive measures of a broad band of neuropsychological functions are then employed, and other important variables related to development are scaled and treated by statistical techniques appropriate and powerful enough to segregate the effect of lead, the long debated question of low level lead effects should not prove permanently refractory to inquiry.

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DISCUSSION

GUINEE (U.S.A.)

You used the phrases "range of chemical lead poisoning" and levels "associated with toxicity".

Are you implying that the 20% of the children in this range, all had some type of clinical symptoms?

NEEDLEMAN (U.S.A.)

My exact language was that "nineteen percent of the black students ... had dentine levels in the range associated with frank lead poisoning". We have tested 16 subjects who had been treated for lead poisoning. The lowest dentine level was 292 µg/g; the highest was over 800 µg/g. Nineteen percent of the black subjects had dentine levels within that range. Some of these "asymptomatic" children had levels greater than 800 µg/g. Eight percent of the white students also had dentine levels in that range.

ALBERT (U.S.A.)

Were blood leads determined in children who had elevated dentine lead levels and was there a correlation?

NEEDLEMAN (U.S.A.)

While there is little relationship to blood lead level at age 8 (about two years after tooth shedding), in another study a close relationship between blood lead level at the time of shedding was found.

ALESSIO (Italy)

I should like to know if in the cases he has examined the author has looked for a correlation between dentine lead concentration and levels of lead in the urine after chelate drugs have been administered.

NEEDLEMAN (U.S.A.)

I am sorry but the urinary data was not available to us though examination of hospital charts.

CARNOW (U.S.A.)

Did you examine deciduous teeth pulled out earlier than 6 years and was there any age related difference?

Comment: There was a third El Paso study - the first one carried out by our group with the city of El Paso. Our findings paralled those of the CDC and included anaemia, symptoms suggesting neurophysiologic dysfunction and so on. The statement to a previous speaker regarding the need for removing lead from food "since studies show no damage" must be carefully examined and such judgments made on the basis of the total literature and not on any single study - particularly one of variance with others which examined the same population.

NEEDLEMAN (U.S.A.)

We did have an opportunity to mesure lead levels in some of these teeth and could find no age related difference.

ZIELHUIS (Netherlands)

What is the effect of caries on Pb in tooth? What is the intraindividual variability of Pb in different teeth in the same child?

What is the timelag between : Pb in blood → Pb in teeth?

NEEDLEMAN (U.S.A.)

We scaled dental caries and could find no relationship to lead concentration. We also studied different teeth from the same mouth and found general agreement as to lead concentration between teeth. The critical factor appears to be the accuracy with which the slice is taken from the central plane. I have no data on the time relationship between blood and tooth lead levels.

KAMINSKI (U.S.A.)

It is true that teeth, as any other calcified organ in the body can act as a storage area for lead, but I do not believe that one can distinguish by this method whether the elevation of the lead content in the deciduous teeth was the result of a continuous low level exposure to lead or as a result of infrequent but massive exposure to lead such as may occur in Pica.

Were there any air lead determinations made to show the correlation to which you have referred to, that is automobile traffic or industrial sources of lead.

NEEDLEMAN (U.S.A.)

I agree that our analysis does not discriminate between acute high level exposure, and chronic lower level exposure. We are working on that problem. We did not have air lead determinations but the small number of dust lead determinations (table III) shows levels as high in the western part of District 5 as those taken adjacent of the paint factory. These suggest to me at least that another source is bringing the lead levels in the western half to meet those near the stationary source. The automobile and truck traffic in the western half is extremely heavy.

McCABE (U.S.A.)

You have reported an interesting tool, i.e., tooth lead analysis, that was used to survey a high-risk population of children who were exposed to excess amounts of lead from their dwelling units or from an industrial source.

I would like to know however, your rational for relating any possible deliterious effect in these children to their measured levels of tooth lead.

NEEDLEMAN (U.S.A.)

We have presented prevalence exposure data today based upon dentive lead concentration. Having identified these high lead children, one can now measure the relationship of exposure to deficit by traditional epidemiological techniques. These would be essentially similar to studies employing blood lead levels, but the advantage of using dental tissue is that one can study older children and still have a measure of their earlier exposure during critical periods of brain development.

LEAD ETHANOL AND δ-AMINOLAEVULINIC ACID DEHYDRATASE

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ABSTRACT

Delta-aminolaevulinic acid (ALA) dehydratase activity is depressed by lead and by ethanol both "in vivo" in man and animals and "in vitro". When erythrocyte ALA dehydratase activity was measured in normal subjects and compared with blood lead values, a highly significant negative exponential regression curve was obtained. It was found, however, that in a group of alcoholics, in whom blood ethanol levels were not measurable, there was no relationship between erythrocyte ALA dehydratase activity and blood lead levels, even when paired for ALA dehydratase activities with the control values.

In rats, the combination of lead and ethanol administration gave erythrocyte ALA dehydratase activities significantly greater than the separate depressed levels of activity but significantly lower than the control activity, "in vitro" experiments on rat liver gave similar results.

It is suggested that these results are linked through the cofactor reduced Glutathione which potentiates ALA dehydratase activity and is depressed during lead exposure yet elevated by ethanol administration.

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1. Introduction It has long been accepted that lead has a direct effect upon Haem biosynthesis (Goldberg, 1972). In particular lead has been shown, both by direct studies of activity and also by measurement of excreted urinary δ aminolaevulinic acid (ALA), to markedly inhibit ALA dehydratase (EC, 4,2,1, 24) the second enzyme in the haem biosynthetic pathway. Similarly acute ethanol intoxication depresses the activity of ALA dehydratase. When normal subjects consumed whisky, blood ALA dehydratase activity was depressed as blood ethanol concentrations rose to a maximum and rose 'pari-passu' with depression of blood ethanol levels (Moore et al, 1971). In chronic alcoholics blood ALA dehydratase activity is also depressed even when blood ethanol concentrations are zero, normal activity only being regained after about one week's abstention from ethanol (Krasner et al, 1974).

The present studies have been designed to examine the effects of these two factors on ALA dehydratase activity separately and together.

2. Materials and Methods (i) Human Studies These were carried out on a group of alcoholics attending an outpatient clinic at Stobhill Hospital. None were receiving any medication for their condition or for associated conditions.
(ii) Animal Studies were carried out on male Sprague Dawley rats weighing 200g. fed with diet 41, to which had been added 1 per cent lead acetate, for five weeks. At the end of this time, two hours prior to death by cervical dislocation, half of this group were injected with 2ml 30-per cent ethanol/saline solution; the other half being injected with an isocaloric glucose/saline solution. In addition, a second group of 12 animals on diet 41 without added lead acetate were separated into two groups and injected in a similar manner. After death, blood was collected from the carotid artery in heparinised bottles and the liver was excised.

In all experiments blood ALA dehydratase activity was measured by the CEC Standardised Method (1973) and tissue ALA dehydratase by the method of Moore et al, 1971, the results being expressed in both cases as nmol ALA/min/mlRBC or /g wet weight rat liver (Units ALA D). Lead levels were measured by Graphite Furnace Atomic Absorption spectrophotometry and blood ethanol values by gas liquid chromatography.

Results are expressed as Mean ± Standard Deviation.

3. Results (i) Human Studies A group of 88 alcoholic patients was examined together with a group of 22 normal volunteers. All gave blood samples with informed consent. Blood ALA dehydratase activity, lead and ethanol values were measured in all patients (Table 1). In all cases blood ethanol levels were negative. In the control group, a highly significant regression line was obtained with equation:-

$$ALA.D = 57.2e^{-0.582\ PbB}.$$

In the alcoholic group, there was no correlation between blood lead and ALA dehydratase although both mean lead levels were higher and mean ALA dehydratase activities lower (Table 1). When alcoholics with greater than the lower limit of normal for ALA D activity (25 units) were compared with the normal group, there was no significant difference in mean ALA dehydratase activity or in mean blood lead levels. There was however no significant correlation between these values in the alcoholics.

TABLE 1 ALA DEHYDRATASE AND LEAD LEVELS IN ALCOHOLICS

	Number of Patients	Erythrocyte ALA.D Activity (Units)	Blood Lead (μg/100ml)	Exponential regression coefficient (r) (ALA.D v PbB)
Normals	22	32.6 ± 9.1	21.3 ± 5.2	-0.582
Alcoholics	88	18.2 ± 12.6*	29.6 ± 11.6*	-0.060
Alcoholics (ALA.D > 25)	23	28.3 ± 6.6	21.5 ± 6.4	-0.240

* $P < 0.01$ with respect to normal values

(ii) Animal Studies In the 'in vivo' studies significant depressions of erythrocyte ALA dehydratase were observed at mean blood lead concentrations of 132 ± 38 μg/100ml whole blood and at mean blood ethanol concentrations of 194 ± 41 mg/100ml whole blood. When these were combined at similar concentrations of 123 ± 60 μg lead/100ml whole blood and 189 ± 34 mg ethanol per 100 ml whole blood, the activity of erythrocyte ALA dehydratase was significantly elevated over the two separate depressed activities though lower than the initial control value (Table 11).

TABLE 11 THE EFFECT OF LEAD AND ETHANOL ON RAT ERYTHROCYTE ALA DEHYDRATASE

	n	Blood Ethanol (mg/100ml)	Blood Lead (µg/100ml)	Erythrocyte ALA Dehydratase (Units)
Group 1 (Control)	10	0	10.4 ± 8.3	7.26 ± 3.25
Group 2 (Ethanol)	10	194 ± 41	12.8 ± 7.9	5.13 ± 3.35*
Group 3 (Lead)	10	0	132 ± 38*	4.97 ± 3.49*
Group 4 (Lead + Ethanol)	10	189 ± 34	123 ± 60*	6.41 ± 3.55+

Significance with respect to control:- * $P < 0.001$
+ $P < 0.05$

In a subsequent experiment in vitro, with rat liver as a source of the enzyme, it was found that ALA dehydratase activity was again depressed by both ethanol and lead separately but elevated by these compounds together (Figure 1), although still lower than the control value, at all times,

4. Discussion In these experiments it has been shown firstly that ALA dehydratase activity normally a good bioanalytical measure of lead exposure, ceases to be so in alcoholics. In addition, it has been shown that depressions of ALA dehydratase by both lead and ethanol separately are partially reversed when these are combined both 'in vitro' and 'in vivo'. The linking factor in these experiments seems to be the levels of reduced glutathione (GSH) in the system. Lead is known to bind to sulphydryl groups and other workers have shown that addition of GSH to systems measuring ALA dehydratase minimises the depression of activity due to lead (De Barriero, 1969). Conversely, although GSH is a cofactor for ALA dehydratase, excessive concentration of GSH lowers the activity of ALA dehydratase (Moore et al, 1971). Ethanol oxidation substantially alters the redox potential of the cell, and in doing so, alters the ratio of reduced to oxidised glutathione (GSH/GSSG) in the cell. It is therefore suggested that although lead depresses GSH levels and thus lowers ALA dehydratase activity, and ethanol raises GSH levels and lowers activities, these two in conjunction, act in opposite directions and lead to an effective maintenance of activity. (Fig. 2)

Figure 1 : THE EFFECT OF ETHANOL ON RAT HEPATIC ALA DEHYDRATASE ACTIVITY 'IN VITRO' IN THE PRESENCE OF A FIXED CONCENTRATION OF LEAD

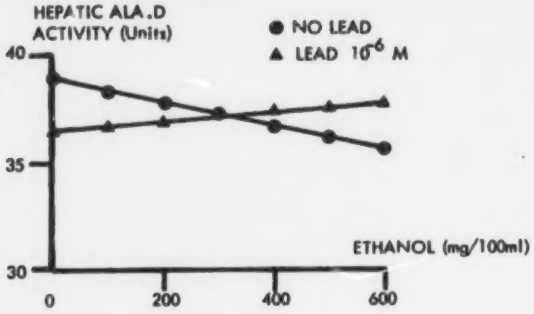
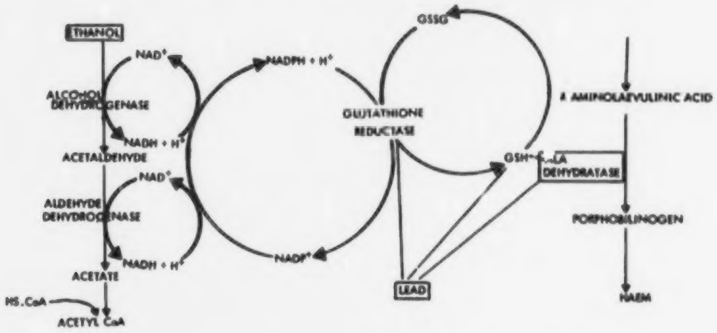


Figure 2 : Inter-related effets of lead and ethanol on GSH and ALA.D activities.



It is suggested therefore that in the use of ALA dehydratase as a bioanalytical measure of lead exposure or as a measure of ethanol consumption in alcoholism, cognisance be taken of the effects of ethanol and lead respectively.

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THE USE OF TRACER TECHNIQUES AND ENVIRONMENTAL SOURCES FOR EVALUATION OF THE LEAD PROBLEM IN CHILDREN

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ABSTRACT

Many literature references cite the fact that, especially in the United States, children living in deteriorated housing have a problem with lead poisoning. Eating leaded paint has been recognized as the prime cause of elevated blood leads. Recently, there has been speculation, originally by the U.S.EPA, that children may eat dust containing lead exhausted from automobiles and that this may contribute significantly to the childhood lead problem. This paper compares lead in paint on houses with lead from automobiles as a source of lead in soil and proposes a new tracer technique to evaluate whether children eat dust and air-suspended particulate.

To determine the lead source in soil, we analyzed lead in soil around old painted houses in the city and in rural areas remote from traffic. The concentration and distribution of lead around the city and rural houses was nearly identical, with the highest concentrations near the houses. Thus, it is clear that lead in the soil was due to paint and that lead antiknocks were not a significant contributor.

To estimate the ingestion of dust and airborne particulate, a naturally occurring radioactive tracer (lead-210) was used to

determine the relative amounts of dust and other lead-containing materials, such as paint, eaten by the children. Analysis of paint samples showed very low levels of lead-210, while dust samples contained at least 100 times as much lead 210. Stable lead and lead-210 were measured in the excreta of children suspected of having elevated lead body burdens and of children living in good housing where lead poisoning is not a problem. The "high lead" children excreted much more stable lead than the "normal" children. Despite the differences in stable lead excretion between the groups, excretion of lead-210 was essentially the same. These results do not support the hypothesis that these children eat dust and air-suspended particulate.

1. Introduction

Lead poisoning in children, especially those under 4 years old, is prevalent in areas of our larger cities where housing has deteriorated. Historically, almost all cases of lead poisoning in children have been attributed to eating paint chips with a high lead content [1,2]. Recently, Fine et al [3], in a study in Illinois, found that elevated blood lead was also common in smaller cities. In their study, they screened children from poor housing areas and concluded that the lead problem is not confined to large cities. The common factor between the large and small cities was deteriorated housing.

Recently, it has been speculated that soil, dust, and air-suspended particulates may also contribute to the lead problem in children [4].

This paper considers two aspects of the childhood lead problem:

1. The major sources of lead in soil around houses where children play.
2. The extent to which children ingest dust and air-suspended particulates.

2. Lead in Soil

Past studies have shown that deteriorating paint is an important source of elevated lead in soil. Hardy et al [5], have reported on analysis of lead in soil near a barn remote from traffic in rural Lincoln, Massachusetts. Soil next to the barn contained 2000 ppm of lead, and the level was 160 ppm 20 feet from the barn. Bertinuson and Clark [6], concluded that urban housing appears to be a larger contributor to elevated lead in soil than emissions from vehicular exhaust. Fairey and Gray [7], found high concentrations of lead in soil in yards, with the highest concentrations generally near the houses. They attributed this lead to paint and ashes.

In our study, we sampled soil at 9 sites around each of 18 frame houses in widely scattered urban areas of Detroit. These areas are characterized by old houses that had been painted with lead-based paint, presumably for many years. Analysis confirmed that all houses were coated with paint containing lead. Similarly, soil samples were taken at 9 sites around each of 18 houses of brick construction. In all cases, these houses had painted trim. For each urban house, surface samples were taken at the following locations:

Dirt in the street gutter.

Soil between the sidewalk and curb adjacent to the curb.

Soil two feet toward the house from the front sidewalk.

Soil on each of the four sides of the house within two feet of the house.

Soil ten feet from the house in the front and back yards.

We also sampled soil around 7 farmhouses in an area remote from traffic located about 30 miles from the nearest city and about 50 miles north of Detroit. Samples were taken from the surface in the same manner as used for the urban samples. Table I is a summary of the data from this survey.

Table I

Lead in Soil

	Lead in Soil, ug Pb/g dry soil				
Sampling Location	Within 2 Feet of House	10 Feet from House	Near Sidewalk	Curb	Gutter
Urban Frame	2010	436	627	572	966
Urban Brick	468	178	324	612	1213
Rural Frame	2529	609	-	-	-
Barn*	2000	570	-	-	-

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* From Hardy et al [5]

Lead in soil within 2 feet of the urban frame houses averaged 2010 ppm, with no obvious bias for front, sides, or back. Lead in soil in the middle of the yards averaged 436 ppm, and again there was no bias toward front or back. The distribution around the brick houses was similar, but the lead levels were lower. The average concentration within 2 feet of the house was 468 ppm, with 156 ppm in the front yard and 200 ppm in the back yard. As with the frame houses, there was no evidence of higher concentrations in the front yard compared to the back. The data for lead in soil around the rural farmhouses are very similar to those for the urban frame houses. The average lead concentration was 2529 ppm within 2 feet of the farmhouses and 609 ppm at 10 feet.

The comparison indicates that most of the lead in soil is due to paint, based on the following reasoning. The lead in soil within 2 feet of the frame houses in the city averages just over 2000 ppm. The lead in soil 10 feet from these houses averages over 400 ppm and is similar in the front and back yard. If vehicular traffic were a significant source of lead, the front yard would contain more lead than the back. Since these data at 2 feet and 10 feet from the house are similar to our data from frame houses in rural areas and to the data of Hardy et al [5], it is clear that traffic is not contributing significantly to lead in the soil in the yards of the painted frame houses.

This conclusion is supported by the data on lead in soil around the brick houses. The lead in soil within 2 feet of these brick houses is more than double that at 10 feet, indicating that the painted trim of the house is the prime source. As expected, the much smaller painted surfaces of the brick houses result in much lower lead concentrations near these houses than those near the painted frame houses. As with the painted frame houses, the concentrations 10 feet from the houses are similar in the front and back yards. Here again, it is evident that traffic does not have a significant effect. The lead in the street gutter was similar for both brick and frame houses. Thus, all evidence points to paint as the prime source of elevated lead in the yards, where the children would be most likely to play.

3. Lead in Dust

The data from the first part of the study show that paint is the major source of lead in soil around the houses where children usually play. The role of lead in air-suspended particles and in fallout dust must be considered separately, since lead in gasoline significantly contributes to the lead content of air-suspended particles and fallout dust.

To distinguish between the leaded paint a child might eat and the contribution of lead he might receive from eating dust, it is necessary to find a material that is present in dust but not in paint.

Lead-210, a naturally occurring radioactive isotope of lead, is a useful tracer for this purpose. It is generated from radon, which is present in the soil. Part of the radon escapes to the air and part stays in the soil. The radon disintegrates with a half life of 3 days to produce lead-210, which has a half life of 22 years [8]. Because it is present in the atmosphere, fallout dust is enriched in lead-210, while paint has very low concentrations of lead-210. The topmost layer of soil is depleted as the radon escapes, but rain brings the lead-210 back to earth where the soil's topmost layer retains the isotope. Thus, the concentration of the isotope is relatively constant with depth.

We found concentrations of lead-210 and stable lead in nonfood materials as shown in Table II. These results show clearly that ingestion of paint will add little lead-210 to the daily burden, while ingestion of dust and soil can quite readily be detected.

Table II

Lead and Lead-210 in Nonfood Materials
(Range of Values)

	Stable Lead, Percent	Lead-210, pCi/g	Ratio, pCi Lead-210/µg Stable Lead
Paint Chips	1-12	0.005-0.07	4×10^{-8} - 7×10^{-6}
Urban Fallout Particulate	1.7-3.7	100-600	3×10^{-3} - 4×10^{-2}
Fallout Dust	0.10-0.19	3-39	2×10^{-3} - 4×10^{-2}
Yard Soil	0.01-0.50	0.2-1.3	4×10^{-5} - 1×10^{-2}
Street Dirt	0.03-0.30	0.7-4.6	2×10^{-4} - 2×10^{-2}

The concept was to use lead-210 as a tracer to determine the amount of dust and perhaps the amount of soil eaten daily by a child. As lead and lead-210 are absorbed poorly in the gut, an estimate of the lead and lead-210 ingested can be made from analyses of fecal matter.

If a child has a high level of stable lead in his fecal matter and a normal level of lead-210, we would conclude that the lead elevation is a result of eating paint. However, if both the stable lead and lead-210 are high in the fecal matter, we would conclude that dust and soil are contributors in addition to paint.

At Children's Hospital of Michigan, we collected urine and fecal samples from children who were suspected of having elevated body burdens of lead. The evidence used was one or all of the following:

1. X-ray showed radio opaque materials in the gut.
2. History of pica.
3. Elevated blood lead.
4. X-ray showed lead lines in the long bones.

Fecal and urine samples were taken from eight such children. These children were one to three years old and all had exhibited pica tendencies. All stool and urine were collected separately during the first 24 hours after admission to the hospital to insure samples representative of the child's usual environment, not that of the hospital. To provide a baseline, combined stool and urine samples were taken from 10 children of the same age level (1 to 3 years) who lived in good housing in Detroit and its suburbs where lead poisoning is not a problem. All samples were collected during the late spring and early summer months. These samples were analyzed for stable lead and lead-210.

Table III shows the lead and lead-210 data for the normal children and the "high lead" children. The normal children averaged 4 micrograms lead per gram dry feces, with a range of 2 to 7. Of the eight children suspected of having elevated lead body burdens, two had fecal lead values (4 and 7 micrograms lead) within the normal range. However, the remaining six were 4 to 400 times higher. Despite these differences in stable lead excretion between the two groups, the groups were essentially identical in the lead-210 content of their feces. The "high lead" children averaged 0.040 picocurie lead-210 per gram dry feces, while the normal children averaged 0.044.

Table III

Lead and Lead-210 in Excreta

Normal Children		Hospitalized Children	
Stable Lead, ug/g dry	Lead-210, pCi/g dry	Stable Lead, ug/g dry	Lead-210, pCi/g dry
3	0.019	19	0.046
2	0.021	20	0.018
3	0.027	18	0.024
7	0.120	49	0.047
7	0.087	4	0.050
3	0.041	7	0.039
3	0.026	40	0.063
5	0.028	1640	0.037
4	0.044		
4	0.024		
Avg.	0.044	Avg.	0.040

Statistical examination of the lead-210 data show that they are log normally distributed and that there is no statistical difference in the concentration of lead-210 between the two groups. The results of this experiment do not support the hypothesis that these young "high lead" children eat dust.

An examination on the ratio of lead-210 to stable lead in the diet of these children clearly shows the difference between the normal and "high lead" children. As a baseline, the ratio in food is about 5×10^{-3} to 20×10^{-3} pCi lead-210/µg lead. For the normal children, the ratio varied from 6×10^{-3} to 17×10^{-3}. For those children who were clearly ingesting lead, the ratio varied from 2×10^{-5} to 2×10^{-3}. This ratio of 10^{-5} is similar to that for a paint containing 5 % lead and 0.05 pCi lead-210/g paint if one takes into account that the child received some lead-210 in his food. The lead-210 data for these 18 children can be related to the amount of lead-210 that is normally present in the diet. The normal children in this study excreted an average of 0.67 picocurie of lead-210 per day in 15 g excreta (dry weight). This value agrees very well with an estimation based on lead-210 data of Morse and Welford [9] for adults. They found that adults ingested about 1.4 picocuries of lead-210 per day. Using literature estimates that a child consumes about half the food of an adult [10,11], an intake of 0.7 picocurie of lead-210 per day would be expected for a child.

4. Summary

This report has described the results of a two-part study to determine whether lead emitted from motor vehicles contributes to the lead problem in small children. In the first part, we determined lead in soil around houses in urban areas and rural areas. The data from the urban areas clearly show that the principal cause of elevated lead in the soil in the yards is leaded paint on these houses. These data were confirmed by measurements of lead in soil around farmhouses, which showed lead in soil concentrations as high as any that have been reported in urban soil.

In the second part of the study, we determined whether children ingest measurable amounts of particulate or dustfall. We used a naturally occurring tracer, lead-210, which is present in relatively large amounts in dust but nearly absent from paint. The results showed that these children with pica (and other evidence of high lead intake) and normal children excreted identical amounts of lead-210. Examination of the ratio of lead-210 to stable lead in the feces and in the possible sources indicates that the source of lead in these children was paint. Consequently, dust and air-suspended particulate were not shown to be sources of lead in these urban children.

APPENDIX

Study Techniques

Soil Samples

Soil samples were collected by taking the topmost layer of soil. All soil samples were dried at 100°C overnight. Lead was extracted with hot dilute nitric acid and determined by atomic absorption.

Biological Samples

The samples from the normal children were collected in acid-washed plastic containers for 24 hours by the mother.

Urine and fecal samples from children admitted to Children's Hospital were collected separately in lead-free containers during the first 24 hours after admission.

All fecal and urine samples were weighted and dried at 100°C. The dry weight was recorded and the sample was taken into solution with nitric and perchloric acid. The lead was taken into methylisobutyl ketone and analyzed by atomic absorption.

Lead-210 Analysis

Only a small portion of the methylisobutyl ketone-lead solution was used to determine lead. The remainder was oxidized with nitric acid. After fuming three times with a few ml of HCl, the lead-210 was determined by the method of Black [12].

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